

**nature**

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## The law moves in

A MEETING of the American Association for the Advancement of Science (AAAS) is a hard time for journalists. Somehow one has to find a theme behind it all; not just the anonymous title adopted for this year's gathering in Denver—'Science and Change: Hopes and Dilemmas'—but some ground base on top of which runs the counterpoint of corridor and coffee-shop conversation.

It isn't an easy task. First, there is the preposterous proliferation of sessions about which almost everyone seems to complain. At any one time you have to choose amongst weather modification, Mars, everyday physics, solar energy, energy in the Rockies, polar research, food pests, desert dust, behavioural research, organ transplants, early experience in primates, appropriate technology, cognition, minorities in science, limits to growth, information policy, religious minorities, systems research and the problems of a science academy. Second, scientists tend (as at the British Association) to drop in, cast their pearls and drop out. With some honourable exceptions this leaves little time for long conversations. Third, the rhetoric doesn't help—what do administrators really mean when they talk about addressing the issues through a hopefully ongoing soft-failure mode of evaluation, and assessment within a prioritised problem-solving environment?

And yet there was at least one theme to emerge from it all, and that was the increasing attention that lawyers are paying to the scientific and technological scene. Some of this attention has been in the field of human rights, where the remarkable action of President Carter in writing to Dr Sakharov has raised many hopes amongst both scientists and lawyers. But scientists and lawyers don't always find themselves fighting on the same sides, and several sessions were devoted to the difficult issues of scientific freedom and responsibility, not just in the abstract but with pressing matters right to hand. The Governor of Colorado, Richard Lamm, who came to power not by the conventional political route but through an environmental concern which captured the state's imagination, put the problem remarkably succinctly in a public lecture. He had, he remarked, been surprised to see a session entitled 'Science: the Key to our Political Future'. (He would have been even more surprised if he had attended the session by some of the let-the-scientists-get-on-with-it views expressed.) For Governor Lamm a more appropriate title would have been 'Politics: the Key to our Scientific Future'. And the theme that science and technology must expect to be increasingly circumscribed by politicians, the courts and public opinion

was widely taken up.

It is not only the recombinant DNA question which has aroused some new concerns. The legal position of those working on earthquake prediction is likely to be questioned before long in the courts. The recent Teton Dam disaster in Idaho, subject of an excellent documentary shown, coincidentally, on television during the conference, raises further questions of how much responsibility the scientist can bear for a project that fails. But the most striking example at present was on the AAAS's doorstep in Colorado—weather modification. The western United States is in the grips of a drought and there is little snow on the mountains to provide relief this spring. The Denver Hilton was doing its bit, it announced, by not providing iced water for customers in its restaurants unless they specifically asked for it. A golden opportunity for rain-makers to show their simple yet still highly debated skills. One such person boldly distributed his advertisements around the conference, 'for rain in only 6 days, anywhere, call . . .'. But things are becoming less simple. A whole day was devoted to a scientists/lawyers meeting on the law of weather modification, and the very same day the newspapers announced that if Washington state starts cloud-seeding, its neighbour, Idaho, will go to law to stop it.

There is little doubt that the law in the United States contains many as yet unexplored pitfalls for the scientist. There is a crucial distinction between speech and action in the constitution, which projects (in the most lurid example) the man who curses the flag but not the man who burns it. Likewise scientists seem to have the right to write, say and publish fairly freely, but what they do has no similar protection. The opposition to scientists is bound to grow—one honoured alumna of the University of Colorado, Boulder, told the local newspaper, 'I had a fairly competent position in science and felt impotent to do anything about the directions it was taking. As an attorney, I have a much better shot at controlling what they're doing than I had on the inside'. Scientists had better start facing these questions—not, one hopes, from a guild mentality, nor from the exaggerated stance heard at the conference that all science is being threatened, but simply because a good working relationship with lawyers is going to be necessary for science to flourish in the future.

Several people commented almost nostalgically that AAAS had been the quietest for years. No tomatoes thrown, no bitter squabbling, no police being called in. These days the police have been replaced by lawyers. □



# Heading for harmony?

The debate prompted by genetic manipulation developments is intensifying in Europe. **Chris Sherwell** reports

IN PARIS on Tuesday a committee gathered quietly for its routine monthly meeting to discuss research involving genetic manipulation using recombinant DNA technology. In Brussels last Thursday another committee lodged deep in the labyrinth of the European Commission of the EEC met to discuss the same subject. On Thursday of this week Britain's equivalent body gathers for its own regular monthly session. Within the next week a commission on genetic manipulation in the Netherlands is expected to report its long-awaited findings. And on 15 March a new and potentially crucial group within the European Science Foundation (ESF) convenes its first meeting in Strasbourg.

Plainly, the pace of developments relating to recombinant DNA research in Europe has quickened. The debate is now consuming the time of government officials, science administrators and molecular biologists in at least nine different countries and no less than five international organisations. Indeed, the aforementioned meetings are but the tip of an enormous iceberg, and if activity is symptomatic of decisiveness, the energy now being spent discussing the subject ought inevitably to produce results. For those researchers actually wanting to do the work, however, it has meant long periods of delay, uncertainty and confusion.

The debate itself stems from the development over three years ago of elegant new genetic engineering techniques, the implications of which prompted widespread concern. This spawned the growth of guidelines, specifying conditions under which various types of experiment using the techniques should be done, and the growth of committees to administer them. The result is that the issue has spread well beyond the bounds of the research community immediately involved.

In the United States for example, where the pace has been hottest, the debates freest and public involvement greatest, the National Institutes of Health (NIH) finally published a complex set of guidelines last June. Failure to broaden these voluntary controls now threatens legislation to make them stick (see box), a development which could complicate the European debate.

So far the European debate has proceeded unsensationally, mostly at the national level; progress is varied. International discussions have gone

quietly ahead, both informally among individuals and formally through those bodies which have perceived a role for themselves. The chances of them continuing without rancour could diminish, however, particularly if national differences emerge strongly in certain international forums.

## Country catalogue

Most European countries have in fact trodden broadly similar paths. The examples of the United States and Britain have been important, the latter adding a new word to the English vocabulary: 'geemag'. The Genetic Manipulation Advisory Group (GMAG) was established in Britain last year following recommendations from the Williams working party on genetic manipulation.

Precautions contained in the Williams guidelines, like those in the NIH guidelines, involve both physical and biological containment. The standards of physical containment are more strict under Williams, however, while biological containment receives greater emphasis in the NIH guidelines. Among other differences, the NIH guidelines provide an extensive list of experiments and appropriate containment measures; under Williams GMAG decides matters case by case. GMAG itself had early problems over trade union representation, but later began business and by this week was meeting again (see box).

At least eight other countries in Europe have sought to involve themselves in the same sort of process.

**France.** In France two committees are

directly involved. One, called an 'ethics committee' and having under 10 members, meets spasmodically to discuss the whole subject of genetic manipulation. The other, known as the *Commission de Controle*, was set up in 1975 under the auspices of France's main research body, the *Délégation Générale de la Recherche Scientifique et Technique* (DGRST). It has a membership of about a dozen, most of them with interests in recombinant DNA and related research. Industry and trade unions are not directly involved, though this could change: both are evidently prepared to abide by the *Commission* line.

The *Commission* meets regularly once a month to assess the conditions under which experiments in France are to be performed. It began by using the Asilomar guidelines, the product of the Asilomar conference in California in 1975, and subsequently used the NIH guidelines. More recently it has used both the Williams and NIH guidelines.

Over a period of some 18 months it has considered some 50 applications. Less than 10% have involved the more stringent containment conditions equivalent to the P3 and P4 categories of the NIH guidelines. The number of facilities in France which could now provide such containment is no more than a couple, and those would probably be at the P3 level.

**Germany.** The more fretful search for West German guidelines has produced four drafts, the latest of which was due to be distributed by the end of February, with the hope of producing a final draft by the spring. The government department involved, the Ministry of Research and Technology (BMFT), took over after a somewhat unsuccessful start under the German Research Council (DFG) using NIH guidelines.

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The new national guidelines are expected to follow much the same lines as the NIH and Williams precedents. There will probably be an equivalent to Britain's GMAG with mixed representation. Guidelines will be voluntary, and it is hoped that industry, for the inclusion of which there are apparently no immediate plans, will follow them. There are no national P4 facilities in Germany, and few that would qualify as P3 facilities.

**Switzerland.** The Swiss Academy of Medical Sciences created a standing committee known as the Commission on Experimental Genetics. Headed by Werner Arber, Professor of Microbiology at the University of Basel, it has a membership of about a dozen, consisting of experts in the field and government officials from the health and science and technology ministries. Industry is ostensibly as keen as the universities to see controls implemented, and is represented on the Commission.

The Commission decided recently to follow the NIH guidelines, but this could still change in the future. It has been agreed with government agencies that no legislation is necessary. Researchers applying to the Swiss grant-giving body (*Suisse National Fonds*, SNF) and supplying relevant details would go before the Commission only when the SNF is unhappy; but they will probably be invited to register their work.

**Sweden.** An 11-man "committee concerning research with recombinant DNA" was set up last spring under the auspices of the Natural Science Research Council (NFR), the Medical Research Council (MFR) and the Swedish Cancer Society (RmC). Its chairman, Professor Peter Reichard of the Karolinska Institute, is the appointee on the committee of the NFR, which also appoints two lay representatives—in this case two MPs. The MFR, RmC, the Council for Forestry and Agricultural Research, the Board of Health and Welfare and the Association for the Pharmaceutical Industry each appoint one member; one member jointly represents the Academy of Sciences and the Academy of Engineering Sciences, another jointly represents the National Defence Research Institute and the Board for Technical Development; the Central Organisation for Salaried Employees appoints a representative for technical staff.

The committee will help granting agencies and government authorities to determine safety conditions for the experiments which they fund, and will probably work in the private sector as well. The committee will also advise researchers over safety precautions and help in risk classification, for which a

working group has just been appointed. Researchers will be expected to submit experimental protocols to national advisory committees, which are responsible for specifying the containment measures. The containment procedures proposed are those of Williams, but experiments prohibited under the NIH guidelines, it is suggested, should not be carried out. Local safety committees would be responsible for supervising the measures.

So far the committee has received no applications and no recombinant DNA work is proceeding. The only group in Sweden which has worked in the field is at Uppsala under Professor Lennart Philipson, the MFR's appointee on the committee. His work is in abeyance, and he has yet to submit an application. The group has applied to have two new P3 laboratories built, but finance for these is not yet assured.

**Denmark.** Denmark has two committees. One, headed by Dr Kjeld Marcker from Aarhus, is an ad hoc committee established by the country's research council. It is examining work being done in Denmark and hopes to decide by the summer whether there ought to be a special research programme. The other is a Committee of Registration with 10 members representing research councils and industry but not trade unions. Its concern is with safety aspects, and it has been operating only a few months. The precise course it follows depends on the outcome of the first committee's work.

**Holland.** In January of last year, after consultation between the Royal Dutch Academy of Science and the National Health Council on the one hand, and the Ministry of Education and Science and the Ministry of Health on the other, a "Commission on Genetic Manipulation" was formed consisting of experts in the field and chaired by Professor Bootsma of Erasmus University, Rotterdam.

Its task was to compile an inventory of recombinant DNA research being done in the country and to advise laboratories on safeguards and the authorities on controls. The commission is expected to report its findings within the next two weeks, but there are differences of view about whether these should actually be published. The Dutch began by opting for the NIH guidelines but then switched to the Williams guidelines; now, however, it seems possible that national guidelines will be proposed which are more strict than either.

So far industry has not shown any public interest in the guidelines. Applications have come for six projects, from four universities. Three have

## Legislation for US?

Legislation to control recombinant DNA research in the United States, a prospect which clearly concerns many scientists, is now considered inevitable. Bills have already been introduced into Congress, and in the next few weeks a committee consisting of officials from a number of federal agencies is expected to draft legislation to be submitted to Congress with the backing of the Carter Administration.

The Administration is reluctantly being pushed into drafting its own bill because if it doesn't, it will invite the prospect of harsher legislation and inconsistent local controls on recombinant DNA research. At present, the only formal controls on recombinant DNA research in the United States are guidelines issued last June by the National Institutes of Health (NIH), but they apply only to research supported by the federal government, and they are not backed by any enforcement or monitoring mechanism. Industry is not bound by any regulations.

Those apparent weaknesses in the present approach have prompted several state and local governments to consider adopting their own regulations on recombinant DNA research, and the fact that industrial research is not regulated at all has caused considerable public concern. Consequently, an inter-agency committee was established late last year under the chairmanship of NIH Director Donald Fredrickson to examine ways to extend the NIH guidelines to cover all recombinant DNA research in the United States. The committee has tentatively decided that no existing federal law can be used to extend and enforce the guidelines, and that new legislation is required.

That tentative conclusion was conveyed by Fredrickson last week to a group of prominent scientists and university administrators which met at NIH. Though Fredrickson repeatedly noted that no decision has been reached on the substance of the Administration's bill, it would almost certainly be designed simply to turn the NIH guidelines into enforceable regulations. It is also likely to include a clause preempting state and local regulations in an effort to ensure that regulations are uniform throughout the United States. It is not clear which agency would do the enforcing—clearly NIH wouldn't want to be in the position of supporting and regulating the research—but the likelihood is that the Center for Disease Control would be given the responsibility.

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come from the University of Amsterdam, and one from the Free University of Amsterdam, the University of Leiden and the University of Groningen.

**Israel.** A committee of the Israel Academy of Sciences and Humanities, chaired by Professor Leo Sachs of the Weizmann Institute, has recommended the establishment of a national safety committee for recombinant DNA research and of special safety committees at universities and research centres.



The local committees would recommend appropriate safety precautions for every research proposal and then pass the matter to the national body, which has the final word.

The Academy decided that Israeli researchers would in general follow the NIH guidelines but would take into consideration recommendations from other bodies.

#### International catalogue

For Europe's researchers, worry as well as confusion is the product of the growing involvement of an increasing number of international bodies. But the important issue—whether or not there should be harmonisation of guidelines—already seems settled. There were benefits in allowing each country to follow its own path and arrive at its own conclusions, but different precautions in different countries threatened to encourage a concentration of researchers, so harmonisation looked preferable.

Moreover, with the subject a matter for public debate, there was the possibility that if one country applied more stringent controls than another, the difference itself could become an issue. Harmonisation could pose problems,

though, if US researchers are faced with legally enforced regulations and there is public pressure for similar regulations in Europe. Creating a disinterested peer group competent to judge colleagues' work could be difficult enough without the added (if necessary) complication of drafting and enforcing legislation.

The case for harmonisation does not, however, solve the problem of which body has the authority to encourage the move towards it. A look at the organisations involved reveals the difficulty.

**The World Health Organisation (WHO).** The WHO has two bodies actively involved, the WHO Special Programme on Safety Measures in Microbiology and the WHO Environmental Programme. Being global and not confined to Europe, and concerned with the public health and safety aspects of the research, the WHO interest is peripheral as yet, although it has begun consultations, with, for example, Professor S. W. Glover, chairman of the International Microbial Genetics Commission, which has a genetic engineering sub-group.

**The International Council of Scientific**

**Unions (ICSU).** ICSU has recently created a sub-committee known as COGENE, headed by the US microbiologist Bill Whelan. Its first official meeting is in Paris at the end of May. Because it too is part of a global agency, and because it represents international commissions in various disciplines more than national councils, ICSU can acquire an important role outside North America and Western Europe.

It can, for example, involve the East European countries and also the USSR, which is apparently keen to participate in the research under agreed guidelines—the USSR has a committee under Professor Bayev which hopes to produce its own guidelines by the middle of the year. ICSU can also involve important Third World countries, such as India and Nigeria, which cannot be prevented from pursuing the research but which might participate in any programme organised by ICSU on their behalf to help ensure that they take the necessary precautions.

**The European Molecular Biology Organisation (EMBO).** EMBO is based at Heidelberg, where a P4 facility for international use is under construction.

## Britain's GMAG gets going

BRITAIN'S Genetic Manipulation Advisory Group (GMAG) has already given outline approval to more than half a dozen experiments, including one that requires the highest level of containment. For the present, however, the only place where such an experiment can be carried out is Porton, a Ministry of Defence establishment whose future is under scrutiny.

GMAG meets monthly to consider proposals for genetic manipulation experiments which, while awaiting a legal definition, are taken to refer to those in which restriction enzyme fragments of nucleic acids are recombined with the genomes of other organisms in which they are capable of propagation. GMAG's chairman is Sir Gordon Wolstenholme; it has 19 members, of whom only three have a direct interest in recombinant DNA research. Most of the others are biological scientists, but there are four union representatives and two non-scientific academics.

It is GMAG's task to consider any proposals and to state its objections, if any. Proposals (25 copies) are submitted on a formidable form on which full details of the protocol, personnel and facilities have to be provided along with information regarding local safety committees, biological safety officers, medical officers and health monitoring schemes. Proposed experiments fall into four categories, depending on the associated hazards. It is up to the proposers to categorise their own experiments but up to GMAG to ascertain whether the relevant requirements are satisfied.

In the case of the two categories (III and IV) that represent the greatest hazards, GMAG has to carry out a site

inspection of the facilities. This they have already done for the category III facility at the MRC Mammalian Genome Unit in Edinburgh. Professor Peter Walker of that unit expresses himself well satisfied with the visit and its outcome which, pending attention to a few minor problems, should give him the first approved category III facility in Britain. Site visits have also been requested for a proposed category III laboratory at Imperial College and for the Microbiological Research Establishment at Porton. It is on Porton that British short-term hopes for a category IV facility hang—though for now on some rather delicate strings. Porton is the property of the Ministry of Defence and has been the laboratory in which much of Britain's microbiological warfare research has been carried out. As such it has long been unloved by many academics. Now it is also unloved by the Ministry, which recently announced that it no longer has a use for the microbiological laboratories. Since December their future has been uncertain and is now the subject of a confidential report prepared by the Central Policy Review Staff for the Cabinet (see Britain, page 9). A decision is thought to be imminent.

There are bound to be problems if the decision leaves the laboratories under the control of the Ministry of Defence. If that happened, it is known that a faction of GMAG, led by some of the trade union representatives, would be strongly opposed to the use of Porton's facilities for civil genetic manipulation. That could prevent the go-ahead of any category IV experiments, including the one that has already been given outline approval.

until an alternative category IV laboratory could be found. At the moment none are planned but it would not be too far-fetched to suppose that the proposed category III facility currently being completed at the Beatson Institute, Glasgow, could be upgraded if required.

It is Scottish laboratories that have so far taken the lead in applying to GMAG, with their English counterparts being distinctly slow off the mark. Of the expected initial batch of 40–50 applications less than 15 had reached GMAG by its third meeting last month and at least eight of those were from north of the border, mainly from Professor Walker's laboratory.

GMAG has in fact expressed surprise at the paucity of applications and has reminded those concerned that they would be foolish not to present their proposals to the group. At present application to GMAG is voluntary although the UK Health and Safety Commission (HSC) is redrafting its controversial regulations that would make notification compulsory. The original proposals were strongly criticised for being far too wide-ranging, especially as existing health and safety regulations already cover the laboratory hazards of genetic manipulation in the general sense.

The Research Councils have themselves warned of their intention of withdrawing funding of any rogue manipulators. Only industry would therefore be immune from financial sanctions, but the two most interested pharmaceutical companies have already made their intentions clear by submitting proposals to GMAG.

Peter Newmark

It was the first international European body to become involved in guidelines for recombinant DNA research, and has a Standing Advisory Committee on Recombinant DNA chaired by Professor Charles Weissmann. This was set up in January 1976 and held its first meeting in London the following month to discuss whether it was worth elaborating guidelines, other than those available, for Europe as a whole.

At its second meeting, in London last September, the committee compared the NIH and Williams guidelines. It suggested the establishment of national advisory groups to specify containment measures for each experiment on the basis of a detailed protocol submitted to it. The committee specifically recommended against the idea of using some combination of the procedures from the two sets of guidelines. And it advised that experiments forbidden under the NIH guidelines not be carried out.

**The European Science Foundation (ESF).** Established in November 1974, the ESF is made up of 45 national research councils and academies from 18 European countries and aims to create a close-knit community of science and research in Europe. Sweden quickly suggested to the founding committee that it should consider the whole question of genetic manipulation, including its social, legal and ethical aspects. With the EMBO committee able only to provide advice on request, and then only about scientific and technical aspects, the ESF decided in October 1975 to broaden a preparatory working party into an ad hoc committee on Recombinant DNA which could propose through ESF

members whether and what action should be taken at European level.

The committee met three times in 1976 under the chairmanship of Professor Povl Riis of Denmark; members included molecular biologists, physicians and lawyers. Their brief was broad, and they concluded that the recommendations and code of practice of the Williams report should be adopted as the guidelines for recombinant DNA research in Europe. They also recommended that national registries of research should be established and that laboratories should be legally obliged to declare their work to it; laboratories would adhere to agreed guidelines voluntarily, however, and supervision and monitoring would be a national responsibility. National variations, it suggested, should be minimised.

The ESF has now created a new committee made up of representatives of the geemags of its members with the aim of proposing guidelines for Europe. This European Committee on Recombinant DNA will meet for the first time in Strasbourg on 15 March. It will note differences in the practices of various countries and consider prescribing measures for the future.

**The European Commission.** The European Commission, which has a dual role in the EEC of both initiating and implementing Community legislation, finally jumped into the fray in January. Spotting the opportunity provided by a potential need for Community-wide legislation and harmonisation, Directorate General XII (Research, Science and Education), headed by Dr Gunter Schuster, called geemag heads to Brussels on 21 January for "informal consultations". This offended some

sensibilities, not least because Dr Schuster was seeing members of the new ESF committee on which he is himself the EEC's representative.

The Commission is apparently contemplating a directive, the device by which it can request member states to modify and harmonise their legislation. In the case of recombinant DNA research this might involve asking the Nine to ensure that they take the same precautions, but not interfering with the operations of individual geemags. At the January meeting there was no stern objection to the idea, provided a directive was not too specific or detailed.

The worry is not chiefly about Commission interference, although its record in science is less adequate than it might be. Most people recognise that it possesses the authority both to hasten the necessary harmonisation and to incorporate research done in the private sector a common framework. The worry for the moment is related more to the style and timing of the Commission's involvement, which could be self-defeating if it breeds resentment among researchers.

The outcome of the January meeting was presented last week at a meeting in Brussels of the Medical Research Committee, a sub-committee of CREST, the Commission's Scientific and Technical Research Committee. According to the office of the director of the biology programme, the meeting reached no firm conclusions and is due to meet again only in June. That may mean that EEC involvement will remain peripheral for a while yet. If so, the immediate burden of recommending a path for recombinant DNA research in Europe now lies with the ESF. □

## USA

# From Carter via Ford

*Colin Norman reports from Washington on President Carter's proposed budget changes*

AFTER three weeks of frantically sifting through the massive set of budget proposals which President Ford left behind, President Carter last week sent Congress a raft of major and minor amendments. Designed to implement some of Carter's more prominent election promises and political priorities, the proposed budget revisions would undo some of the Ford Administration's parsimony toward health, welfare and housing programmes and add about \$19,400 million to previous estimates of government

spending in the 1978 fiscal year (which begins on 1 October, 1977).

As far as science and technology are concerned, the only areas which figured prominently in campaign rhetoric were energy and military research and development, and consequently they are the only areas greatly affected by Carter's proposed budget revisions. Outside those two fields, Carter has proposed a small addition to Ford's budget for NASA for studies of possible follow-up missions to the Mars Viking Lander project, and a \$5 million increase in the budget of National Institutes of Health (NIH) for research on childhood diseases. Otherwise, the relatively large budget increases for basic research, earthquake

prediction and agricultural research proposed by President Ford have been left untouched.

The proposed revisions to the budget for energy research and development are another matter. Acting on the advice of James Schlesinger, his energy adviser, Carter has scaled down Ford's budget proposals for three long-term energy programmes—the breeder reactor, development of large solar power plants and thermonuclear fusion—and redirected some of the funds towards efforts likely to produce short-term results. The proposals signal a major shift in priorities and indicate that the new Administration is prepared to play a more aggressive role than its predecessor in pushing new technologies, such as solar heaters and electrically powered automobiles, into the market-place. The budget pro-



## Press report

It is now virtually certain that President Carter will name Frank Press, a distinguished geophysicist from the Massachusetts Institute of Technology, as his science adviser and head of the White House Office of Science and Technology Policy (OSTP). Though no official announcement had been made by the end of last week, Press was already at work in the White House, helping to select candidates for other top science posts in the federal government and attending meetings of Carter's senior advisers. Administration sources indicated that a formal announcement was imminent.

The appointment of Press, which was first rumoured a couple of weeks ago, is something of a surprise. Although he is no stranger to the Washington science policy network, he is not generally regarded as part of the inner circle of the scientific establishment. It was, in fact, widely expected that the job would go to Lewis Branscomb, head of research at International Business Machines (IBM), who coordinated a science policy task force for Carter during the campaign. It has been suggested, however, that Branscomb was ruled out because several people with IBM connections had already been appointed to top posts in the Carter Administration, a fact which has attracted some criticism.

Press was recommended to Carter by a number of prominent scientists, particularly Defense Secretary Harold

Brown and Jerome Wiesner, President of MIT, and his appointment has generally been greeted with warm approval in the scientific community. An outstanding scientist, he was elected a member of the National Academy of Sciences at the age of 33. He was Professor of Geophysics at Caltech from 1955 to 1965 and Director of the Seismological Laboratory for much of that time. Since 1965 he has been Chairman of the Department of Earth and Planetary Sciences at MIT.

Among his credentials for the science adviser post is his wide experience in the science and politics of test ban negotiations. He has long been interested in the problem of identifying the seismic waves from underground nuclear explosions and discriminating them from signals from earthquakes. He was a United States delegate to the test ban conference in Geneva in 1960-62, which paved the way for the partial Test Ban Treaty in 1963, and he has been a consultant to the Arms Control and Disarmament Association. In 1968 he was a member of the international working party convened by the Stockholm International Peace Research Institute which reopened the question of monitoring underground weapons tests. Since Carter has frequently promised to see a comprehensive test ban agreement with the Soviet Union, Press's experience in such matters is said to have been a strong point in his favour.

Other relevant Washington experi-

ence includes a stint on the President's Science Advisory Committee (PSAC) from 1961 to 1964, and membership of the National Science Foundation, from 1970 to 1976. He is also Chairman of the National Academy of Sciences' Committee on Scholarly Communication with the People's Republic of China, and has travelled extensively in China in recent years.

Even though Press's administrative burden has grown heavier every year, he has continued to make major and distinguished contributions to geophysics. His early work was concerned with seismic wave propagation in the earth's crust and upper mantle, and this eventually resulted in a book *Elastic Waves in Layered Media* in collaboration with M. Ewing and W. Jardetzky. The book had a great influence on the emerging science of seismology. In the 1960s his interests turned to lunar seismology and he was a member of the Apollo team that discovered moonquakes. In the same period he was also encouraging early US research in earthquake prediction as Chairman of the Earthquake Prediction Panel of the Office of Science and Technology. In the late 1960s he started to work on the problem of inversion of earth data by Monte Carlo methods, and more recently his research has included study of the excitation of the Chandler Wobble by earthquakes. In 1974 he published, with R. Siever, a major geology/geophysics text *Earth*.

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posals, moreover, are only a foretaste of a comprehensive energy policy which Carter has promised to unveil in mid-April.

The budget revisions suggest that the besieged liquid metal fast breeder reactor (LMFBR) programme is in deep trouble. Carter has recommended that nearly \$200 million should be taken out of Ford's budget request for the LMFBR effort next year, a reduction which would still leave \$656 million in the programme, but which would cut spending to below this year's level. A statement published last week by the Energy Research and Development Administration (ERDA), which is said to have been drafted by Schlesinger, announced that the entire LMFBR programme will be subjected to an intensive review, to "assess the role of LMFBRs in the nation's energy future, the timing and pace of the breeder programmes and the timing of any decision on whether the breeder would be a viable energy

option".

The implications are made clear later in the statement:

The President's energy priorities, as reflected in the revision of ERDA's (fiscal year) 1978 budget request, stress conservation and nearer-term supply technologies. These priorities suggest that past plans for expansion of the LMFBR program may no longer be viable. Furthermore, serious questions have been raised about the LMFBR technology and the structure of the current LMFBR program. The energy potential of this option must be weighed against the safety questions associated with the LMFBR and the dangers of nuclear proliferation from plutonium reprocessing needed by LMFBRs.

In particular, the review will focus on ERDA's plans to build a demonstration fast breeder reactor on the Clinch River in Tennessee, a project which represents the next major step in the LMFBR programme in the United States. Construction of the Clinch River plant was scheduled to begin

later this year, but it will now be delayed pending the outcome of the review. Consequently, Carter has suggested that \$85 million be cut from Ford's request for that project alone. No date has been set for completing the review, but it is unlikely that a final decision will be ready in time for inclusion in the energy policy which Carter will unveil in April.

As for solar energy research and development, again, long-term efforts are being cut back to allow more funds to be channelled into programmes likely to produce more immediate results. Accordingly, Carter has scaled down Ford's budget proposal for construction of a pilot-scale solar thermal generating plant, and recommended that the savings be used chiefly to underwrite the costs of producing and testing some 1,300 solar heaters.

The pilot plant project which Carter wants to cut was only given a go-ahead by the Ford Administration on 7 January. It would entail construction



of a 10 MW generating plant in the Mojave Desert in California; the plant would essentially consist of a bank of mirrors concentrating the sun's rays onto a steam generator atop a tower. The project, the costs of which would be shared with electric utilities and government bodies in California, was scheduled for completion in 1980 or 1981. Ford had requested \$65 million for the project next year, but Carter has recommended that the budget be reduced to \$10 million. The original plan was to follow the California plant with another pilot plant a couple of years later, leading eventually to a commercial-scale plant in the late 1990s. No decision has yet been made on how far the project will be stretched out under Carter's energy policies.

Carter's proposal that more funds be pumped into the production and testing of solar heaters represents a marked change from the policies of the Nixon and Ford Administrations. In 1974, Congress passed a bill to provide federal support for up to 5,000 solar heaters to be installed in public and private buildings and tested over a five-year period. The idea was to stimulate the growth of a solar heating industry, but the Ford Administration was opposed to the principle that the federal government should be so directly involved in the market-place, and the legislation consequently was implemented in a relatively half-hearted manner. Carter clearly shares Congress's view that federal support is needed to commercialise new energy technologies.

Similar views show up in Carter's proposals for energy conservation. He has doubled Ford's budget request for research and development on energy-

saving systems, recommending that a total of \$318 million be spent next year. Much of the proposed increase would be devoted to programmes designed to increase the efficiency with which energy is used in industry, buildings and transport, but \$40 million would also be spent on a programme to provide federal subsidies for the production and testing of electrically powered vehicles. Again, that is an effort which Ford had argued should be the responsibility of private industry rather than of the federal government.

As for the fusion programme, Carter has recommended that \$60 million be docked from Ford's request for research on magnetic confinement, leaving \$311 million in the programme. He also suggested that Ford's request for laser fusion studies be reduced from \$142 to \$122 million. Again, the pattern is to decrease support for long-term programmes, but to allow nearer-term efforts to proceed. Thus, the Tokamak Fusion Test Reactor (TFTR), a major facility being built at Princeton University, will be allowed to go ahead, but the completion date would slip by about 6 months, to mid-1981. Similarly, a large magnetic mirror machine planned for the Lawrence Livermore Laboratory would suffer a \$10 million cut, but it would also be allowed to proceed with only a minor slip in schedule.

As far as the addition to NASA's budget is concerned, the Carter Administration has unexpectedly recommended that \$15 million be spent next year on studies of potential missions to Mars as a follow-up to the successful Viking project: Ford had recommended only \$5 million for such efforts. Several candidate missions are under consideration, ranging from

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Keystone

*Energy man: James Schlesinger*

refurbishing the back-up Viking spacecraft, which was held in reserve in case the original Viking mission failed, and launching it in 1984, to construction of a new spacecraft to return samples of Martian soil to Earth. The more ambitious missions would probably not be launched until 1988. In any case, planetary scientists are encouraged by Carter's modest budget addition, seeing it as an expression of Presidential interest in the space programme.

The only other budgetary change of note is the fact that Carter has added only \$5 million to the budget of the National Institutes of Health. Ford's budget request for NIH was relatively parsimonious, providing an increase of only \$40 million, which is insufficient to keep pace with inflation. Congress traditionally adds substantial increases for NIH, however, and the Carter Administration will probably not object if Congress carries on the tradition this year. □

## SEVESO

# Dioxin damage

*Alastair Hay reports on recent developments in Séveso and a possible link connecting dioxin with birth defects.*

OFFICIAL confirmation has now come that at least two of the women who were exposed to tetrachlorodibenzo-*p*-dioxin (dioxin) at Séveso last year have given birth to children with malformations. But the director of health for the Lombardy region, Dr Vittoria Rivolta, is quoted as saying that it is "not yet possible to link the deformations with dioxin contamination". The dioxin was present in the discharge from a trichlorophenol reactor which overheated at the ICMESA chemical plant on July 10.

The first abnormality to be observed was a slight intestinal obstruction in an infant born on 10 January. The mother had apparently eaten garden produce which was heavily contaminated with dioxin. The second case—a "small genital deformation"—was reported on 7 February. But the mother was resident in an area not officially considered to be contaminated by the chemical.

According to Dr Rivolta both infants underwent successful surgery shortly after birth. He added that, in view of the known teratogenic properties of dioxin, the Lombardy regional health department was keeping "a record of all children who are born with deformations from mothers who were in

the Séveso area at the time of the explosion".

The developing human embryo is particularly susceptible to teratogenic agents during the period of organogenesis when tissue and organ differentiation occur. In Man this period generally occurs between gestation days 18–55. The children born on 10 January and 7 February would therefore have been at a stage of foetal development beyond organogenesis. For that reason they might be considered to have been less susceptible to the teratogenic properties of dioxin. However, as one mammalian development scientist put it, "We don't know the teratogenic mechanism of dioxin and we have no idea at which stage of its development a foetus ceases to be at risk to this chemical".

School children in Séveso and its

neighbourhood are among the latest victims of chloracne, the skin complaint which can appear as a result of contact with dioxin. The number of children affected is reported to be about 340. Most of them, it seems, have come into contact with dioxin through contamination of school buildings. Indeed, dioxin contamination of the buildings is considered to be too serious to allow the schools to remain open. Following protests from school teachers and parents the Lombardy authorities have closed six schools in the Séveso municipality; they will remain closed until the work of dioxin decontamination is completed.

The decontamination programme itself has come in for much criticism in Italy. Local residents are reported to be incensed over the delay in cleaning the Séveso area and the Italian Parliament is concerned enough to consider appointing a commission to investigate the cause of the delay.

The Lombardy administration's de-

contamination programme was, in fact, finally approved by parliament only three weeks ago. The proposed plan of action includes the incineration of vegetation, dead animals and polluted topsoil, which is to be removed to a depth of 25–35 cm. This operation should be completed by August, when all the topsoil will have been placed under canvas on a concrete base at a location near the ICMESA plant. The total material to be disposed of is estimated to amount to about 70,000 tons. It will take three years to burn, in a high temperature incinerator which will itself take a year to construct. The whole decontamination programme will, it is estimated, cost £13.5–15 million and will eventually be paid for by Givaudan, the Swiss company which owns the ICMESA plant.

The variability of dioxin contamination has proved a major obstacle for the local authority in formulating its decontamination programme. With some areas considerably more con-

taminated than others, a detailed relief map of the area has been necessary and this has taken time to produce.

Many residents of Séveso are angry that it has taken so long to devise a graded decontamination programme, with the result that the Lombardy regional government now stands accused of gross mismanagement in its handling of the whole affair. But the outbreak of chloracne among Séveso schoolchildren has resulted in the far more serious charge being levelled that the local authority has minimised the dangers of contamination.

These accusations will now be subjects for consideration by the Parliamentary Commission. For the pregnant women of Séveso, however, no inquiry will relieve their anxiety. If a link is established between the recent infants born deformed and dioxin, the hundred-odd women who were in the first trimester of pregnancy on 10 July and who will reach full term during the next two months face a bleak future. □

## POLAND

# Problem solver

*Vera Rich reports on science policy in Poland following the visit to Britain of the Polish Science Minister.*

THE recent visit to Britain of the Polish Minister of Science, Higher Education and Technology, Professor Sylwester Kaliski, was essentially a matter of diplomatic routine. No major changes were expected in the existing programme of Polish-British scientific and technical cooperation and educational exchanges, and the immediate practical outcome amounted to little more than the agreement by both parties that such cooperation is of mutual benefit, should be continued and, where possible, expanded. Nevertheless, the Ministerial visit served to highlight and provide an opportunity for discussing several interesting aspects of current Polish policy.

Professor Kaliski told *Nature* that, in addition to 65 "key" problems, Polish science policy incorporates seven "government problems" to be tackled at top-priority level: water resources, coal mining technology, electronics and integrated circuits, copper, building engineering, cancer, and proteins. Two others, energy (including atomic power engineering) and computerisation, are in preparation. In all these fields there are either already technological exchange agreements between Britain and Poland or else approaches have been made by the Poles for such agreements

(in certain cases, notably integrated circuits and computers, response is limited by the Co-Com embargoes). Most of these problems lie largely in the field of technology; two, however, cancer and proteins, seem to fall within the sphere of research rather than application.

The inclusion of cancer in such a list of problems is notable. Whereas problems like mining technology or computerisation of production seem amenable to state planning with its targets and schedules, there are no hints of a major Polish breakthrough in cancer research that would make any comparable set of targets more than a list of desideration. Indeed, Poland does not seem to have been assigned a special role in the new Comecon oncological project, which hardly suggests a Polish lead in the field. The explanation may be one of national tradition: Mme Skłodowska-Curie, the discoverer of radium, was a Pole, and the very word radium, to Polish ears, is connected with two roots meaning respectively 'joy' and 'good counsel', attributes associated in the popular imagination with the early hopes of radium as a specific weapon against cancer.

In the field of protein resources, current plans envisage an increase in output of one million tonnes per year by 1980. New sources are being sought, including the production of animal feed from petroleum waste and a programme for exploiting the krill re-

sources of the Antarctic. A research ship, the *Professor Siedlecki*, of the Gdynia Maritime Fishing Institute, is at present in the Antarctic carrying out a "scientific reconnaissance" of krill. Last year, the *Professor Siedlecki* brought home krill specimens for investigation by sixteen research institutes in Poland for possible use for human consumption or animal feed.

This year's expedition is testing methods of processing krill. The prospect of krill harvesting on a commercial scale has been causing concern among ecologists in many countries, since krill stands at the head of so many food-chains. Questioned about this, Kaliski stated that at present the krill project was only at the investigation stage and "we cannot tell what its future will be". No ecological problems would arise, he said, for at least ten years. Ultimately, however, there would have to be some kind of international agreement governing the harvesting of krill.

In the current Five-Year Plan, said the Minister, government spending on sciences will be increased by some 70% from 115 thousand million to 200 thousand million zloty. This, he suggested, presumably referring to the involvement of scientists in the current unrest in Poland, is "proof that we don't believe there is a crisis in our country". The financial target is not represented by the budgets to date, which for 1976 and 1977 envisage expenditure on science of 16.6 thousand million and 15.9 thousand million. A considerable increase is to be expected



in the last three years of the plan, if the 200 thousand million target is to be reached.

The Minister said that of the total expenditure on science some 12–15% is allotted to fundamental research, and the rest to applied research projects, the latter being financed half from the central budget and half from industry by means of research contracts. The implementation of such applied research in production is Poland's biggest problem. This statement is true also of the Soviet Union, but whereas Soviet scientists frequently receive official rebuke for not producing the results that industry needs, the problem in Poland, says Kaliski, is that they "often have very good scientific results but no chance of introducing them". Poland's scientists, he explained, "think that if they have obtained a result, that it must be introduced". This was not always feasible under a socialist system;

under capitalism it would always be possible to find some manufacturer who would undertake to introduce a new process simply for profit motives, but in a system of state planning this was not always possible. To test promising innovations at the pilot stage, he pointed out, the Polish Academy of Science possesses a small pilot factory. The main activity of the Academy, however, is fundamental research and "quality potential", rather than the development of individual applications.

Concerning Comecon, Kaliski said that Poland participated principally in mining, computers, electronics and laser physics. In atomic power engineering, the Poles were "not the leading group—but we all contribute". In the Interkosmos space programme, Poland was concerned mainly with on-board computers, the application of space research to cartography and meteorology, the study of plasma *in vacuo*,

magnetic field research, and signal propagation. She was involved in "serious collaboration" in space medicine. Kaliski appeared quite satisfied with the present scheme of financing Comecon research projects by direct contracts between the countries concerned, and showed no interest in the more sophisticated arrangements suggested recently in the Polish journal of international affairs *Sprawy Miedzynarodowe*.

One interesting feature of Kaliski's visit was his meetings with laser physicists. These were not, strictly speaking, in his capacity as Minister. In addition to his governmental duties, Professor Kaliski is head of the Institute of Plasma Physics and Laser Microsynthesis in Warsaw. In an informal lecture which he gave at the Rutherford Laboratory, Chilton, he described the work of the Institute on microthermonuclear synthesis.

## BRITAIN

# More of the same

*The UK Government has published details of its proposed expenditure plans for the next few years and its Defence Estimates for 1977. Chris Sherwell reports*

LAST week's White Paper, *The Government's Expenditure Plans, Volume II* (Cmnd. 6271-II, HMSO, £2.35), which follows publication earlier of a general account of the plans, contains the details of individual expenditure programmes. Twelve months ago the government published its plans for the rest of the decade, and these promised a painful levelling off in the growth of expenditure. Since then the government has announced two rounds of further measures, in July and December; the impact of the latter has yet to be assessed and incorporated into figures for 1979–80 onwards.

Unsurprisingly, the latest plans confirm the trend foreshadowed last year. Because organised research and development in Britain has a large government element, this means that the circumstances under which the country pursues its science effort remain far from happy. Some of the details from the White Paper are contained in Table 1 (see over).

For the Ministry of Defence, which is the government's biggest spender on R&D, the table shows only the trend in the overall budget. Latest plans are available in more detail in the *State-*

*ment on the Defence Estimates 1977* (Cmnd. 6375, HMSO, £1.50). Expected expenditure on the defence research programme in 1977–78 amounts to £123 million, two-thirds of it devoted to "evaluation and exploratory development" on equipment and weapons, the rest devoted to maintaining a base of scientific knowledge and advancing technology. Another £23 million-worth is linked with civil research.

Defence research did not escape the public expenditure axe last year, and the White Paper confirms this trend too. Implementation of the rationalisation of R&D establishments is described as "well advanced with the first major moves now taking place". By the early 1980s four "systems establishments"—sea, land, air and underwater—complemented and supported by eight "technology establishments", will have replaced the 25 original R&D establishments.

With the reviews of future levels of R&D at Porton complete, work on chemical warfare at the Chemical Defence Establishment (CDE) "can be reduced to about two-thirds its present size", the White Paper says; and the future of the Microbiological Research Establishment (MRE), because microbiological research requirements can be met "by a small team of ten scientists" integrated within the CDE, depends "on the scale and range of civil requirements".

Other details from the White Paper on expenditure include:

- Support for the National Physical

Laboratory, the National Engineering Laboratory and Warren Spring Laboratory (the Department of Industry's Industrial Research Establishments), together with department-sponsored work at the Atomic Energy Authority (UKAEA) and the Natural Environment Research Council (NERC), is expected to remain at broadly the same level up to 1980–81. The general industrial research and development programme overall, however, has been reduced by £2 million compared to last year "as a contribution to savings in public expenditure".

- The pattern of funding for R&D in certain aviation fields will be modified if and when the new nationalised industry, British Aerospace, comes into existence with the passage of the Aircraft and Shipbuilding Industries Bill through parliament.

- Reflecting developments in the past year involving the country's nuclear reactor programme, the White Paper says that the provision for R&D by the UKAEA is now "subject to adjustment in the light of the current review" of the programme.

- The Science Budget, which indicates the organised government effort on the civil side in basic sciences, reflects the reductions of last year. Resources devoted by the Science Research Council to 'big science', the White Paper says, will be reduced by about 25% over the survey period. The breakdown for 1976–77 is shown in Table 2.

- In most nationalised industries, but especially electricity and telecommunications, the lower forecasts of investment in fixed assets are the product of revised estimates of demand. □



Table 1 Trends in science-related expenditure of UK government (£ million at 1976 survey prices)

|                                          | 1973-74 | 1974-75 | 1975-76 | 1976-77 | 1977-78 | 1978-79 | 1979-80 <sup>1</sup> | 1980-81 <sup>1</sup> |
|------------------------------------------|---------|---------|---------|---------|---------|---------|----------------------|----------------------|
| Defence budget <sup>2</sup>              | 5,460   | 5,265   | 5,548   | 5,621   | 5,444   | 5,397   | 5,652                | 5,630                |
| Industrial innovations, of which:        | 370     | 359     | 373     | 281     | 224     | 214     | 207                  | 301                  |
| General industrial R & D <sup>3</sup>    | 38      | 39      | 42      | 51      | 56      | 61      | 66                   | 68                   |
| Technological and industrial sponsorship | 6       | 5       | 7       | 8       | 11      | 12      | 12                   | 12                   |
| Aircraft and aero-engine R & D           | 26      | 25      | 19      | 19      | 13      | 12      | 12                   | 12                   |
| Concorde development and production      | 116     | 102     | 81      | 37      | 34      | 28      | 19                   | 12                   |
| RB211 and other aircraft and engine      | 64      | 76      | 92      | 11      | -11     | -5      | -6                   | -6                   |
| Space                                    | 18      | 22      | 25      | 30      | 25      | 20      | 20                   | 20                   |
| Nuclear                                  | 102     | 90      | 107     | 127     | 97      | 86      | 83                   | 83                   |
| Research councils <sup>4</sup>           | 229     | 232     | 235     | 217     | 216     | 215     | 217                  | 215                  |
| Expenditure on fixed assets by:          |         |         |         |         |         |         |                      |                      |
| National Coal Board                      | 122     | 200     | 241     | 293     | 332     | 373     | 360                  | 377                  |
| Electricity Council and Boards           | 657     | 667     | 699     | 704     | 646     | 622     | 586                  | 570                  |
| South of Scotland Electricity Board      | 109     | 88      | 73      | 59      | 45      | 66      | 97                   | 132                  |
| British Gas Corporation                  | 182     | 267     | 368     | 259     | 233     | 307     | 345                  | 345                  |
| British National Oil Corporation         | —       | —       | 53      | 148     | 173     | 210     | 255                  | 330                  |
| Telecommunications (Post Office)         | 1,004   | 943     | 916     | 917     | 865     | 906     | 881                  | 901                  |
| Health and Safety at Work <sup>5</sup>   | 15      | 13      | 26      | 30      | 35      | 33      | 34                   | 34                   |

<sup>1</sup>Figures shown are those of last year's survey.<sup>2</sup>About 12% of last year's defence budget went to R & D.<sup>3</sup>Includes the Department of Industry's Industrial Research Establishments and department sponsored work at the UKAEA and NERC.<sup>4</sup>See Table 2 for pattern of each Council's Expenditure in 1976-77.<sup>5</sup>New category, covering allocation for activities of Health and Safety Commission and Executive under the 1974 Health and Safety at Work Act.Source: *The Government's Expenditure Plans, Volume II* (Cmd. 6721-II, HMSO, £2.35).

Table 2 Pattern of Council spending for 1976-77 Science Budget (£ millions)

|                                                | Agricultural<br>Research<br>Council | Medical<br>Research<br>Council | Natural<br>Environment<br>Research<br>Council | Science<br>Research<br>Council | Social Science<br>Research<br>Council | Total        |
|------------------------------------------------|-------------------------------------|--------------------------------|-----------------------------------------------|--------------------------------|---------------------------------------|--------------|
| Research grants and contracts                  | 1.5                                 | 11.0                           | 2.2                                           | 26.6                           | 4.1                                   | 45.4         |
| Research units and establishments              | 6.8                                 | 20.6                           | 17.0                                          | 40.8                           | 0.7                                   | 85.9         |
| Research Council grant-aided institutes        | 9.1                                 | 0.0                            | 2.0                                           | 0.0                            | 0.0                                   | 11.1         |
| Postgraduate awards                            | 0.1                                 | 2.5                            | 1.9                                           | 11.8                           | 5.0                                   | 21.3         |
| International subscriptions                    | 0.0                                 | 1.1                            | 0.0                                           | 31.5                           | 0.0                                   | 32.6         |
| Centrally supported schemes and administration | 0.8                                 | 2.2                            | 2.9                                           | 6.5                            | 1.4                                   | 13.8         |
| <b>Total</b>                                   | <b>18.3</b>                         | <b>37.4</b>                    | <b>26.0</b>                                   | <b>117.2</b>                   | <b>11.2</b>                           | <b>210.1</b> |

Table does not include expenditure by the Natural History Museum and the Royal Society (£8 million).

Source: *The Government's Expenditure Plans, Volume II*

## BANGLADESH

● India's construction of a barrage across the Ganges river at Farakka, about 18 km upstream from the Bangladesh border, is diverting water from the Ganges into the Bhagirathy-Hooghly river. The aim is to flush the silt off the river bed and so keep the Calcutta Port navigable throughout the year. But the project poses a threat for Bangladesh, a densely populated state on a delta whose main artery is the Ganges.

It is estimated that the water from the Ganges and its tributaries serves about 37% of the area of Bangladesh in which about 25 million or nearly one-third of the total population live. About 40,000 cusecs of the Ganges water are redirected into the Hooghly river in dry months, and the effects of such diversion could be severe. Irrigation of 400,000 acres of land dependent on Ganges water was noticeably affected in the last dry season.

According to recent statistics published by Bangladesh Water Development Board, the average discharge of

the Ganges at Hardinge Bridge during the last 10 days of December 1976 stood at 74,000 cusecs, as against 47,200 cusecs during the corresponding period of 1975. Before construction of the barrage the flow in this period varied from 118,000 cusecs to 167,000 cusecs; the average flow during March is normally 74,000 cusecs.

Particularly worrying are the rise in the salinity of the water and the extent of saline intrusion into the land from sea. The index of salinity in the Bhairab river went up to 13,600 micromhos per cm<sup>2</sup> in April last year, compared to the historical average of 500 micromhos. The saline penetration inland advanced from the normal 270 km to about 440 km from coast, affecting an area of 6,820 km<sup>2</sup> beyond the limits of past years. The salinity of the Ganges water has since recorded a further rise. In some parts, particularly in Bhairab river, the salinity index has exceeded 15,000 micromhos per cm<sup>2</sup>; it may rise further next spring.

The reduced water flow and in-

creased salinity will in addition have deleterious effects on the forest resources of Bangladesh: Sunderbans, the largest forest reserve near the coastal belt, faces the possibility of thinning out and an ecological imbalance for men and animals there. The ecology of aquatic life is also adversely affected: the depletion of water levels in dry months and enhanced salinity have affected the fish population at the key landing sites, and there could be long term effects on spawning cycles, fish size and variety.

● Bangladesh has been elected a member to the 58-member Governing Council of the United Nations Environment Programme (UNEP). The council is one of the semi-autonomous special bodies of the United Nations with headquarters at Nairobi. Asian states have 13 seats in the body; other countries from the Asian Group elected together with Bangladesh are China, Philippines, Indonesia and Syria.

M. Kabir

## IN BRIEF

**Spacemen return**

The latest crew of the Soviet Salyut 5 space station have returned safely after an 18-day flight. The programme of the mission was the now-familiar mixture of astrophysical and geophysical observations "in the interests of the national economy", the testing of on-board instruments and systems, and medical observations. The results of one of the medical experiments, which investigated the threshold sensitivity of the vestibular apparatus to electrical stimuli under conditions of weightlessness, will be used to compile criteria for the selection of future cosmonauts.

Japan has meanwhile launched her first geostationary satellite, on 23 February from the Tanegashima Island Space Centre. Although it is Japan's tenth satellite, it is the first to remain in a geostationary orbit and should bring advances in communications and resources surveying.

**Change in Grenoble**

Dr John White took over as director of the Institut Laue-Langevin in Grenoble this week following the departure of Professor Rudolf Mossbauer for a teaching chair at the University of Munich. White's place as assistant director at the Anglo-French-German high flux reactor is taken by Tasso Springer, from the Centre for Nuclear Studies in Julich, West Germany.

**EEC geothermal programme**

The European Commission is asking for proposals for research and development for the second stage of its geothermal energy programme. The programme, first announced in August 1975, embraces the acquisition of new geothermal data, prospecting methods, and energy from hot water sources, steam sources and hot dry rocks.

Five UK proposals were accepted for the first stage of the programme, in-

cluding one from the CEEB and two from the Natural Environment Research Council. The EEC will provide about half the cost of the projects involved.

**UK reactor choice**

Sir Alan Cottrell, the UK government's former chief scientific adviser whose questions about the reliability of the steel pressure vessel of the pressurised light water reactor (PWR) prompted a massive study, has again made public his views about the choice of reactor question. In a letter to the *Financial Times* last week he wrote that Britain "would have little to gain and a lot to lose" by choosing the PWR; he also remained doubtful whether it was right to have to rely for safety on human standards in construction, supervision and operation. He described the merits of SGHWR/Candu and AGR systems as "fairly evenly balanced".

THE most successful and long-running serial put out on the radio by the British Broadcasting Corporation is *The Archers*, a programme dealing with a farming community in the imaginary village of Ambridge in the midlands of England. Topics of current interest are often introduced: thus Phil Archer, a vigorous and progressive farmer, decides to go on a management course organised by the official Agricultural Training Board. His wife is only being mildly ironical when she says that this will teach him to "avoid friction and frustration with the men, teach a logical approach to human problems, improve their job performance, create team spirit and good morale, and develop effective face to face communication". The even more successful farmer to whom this was said replies: "It won't leave much time for farming". This episode may appeal to the many scientists who are being pressurised to attend similar courses, organised by the training branches of government departments and research councils. They don't leave much time for research.

Most people would agree that many branches of science are developing so rapidly that it would be easy to get out of date, and that some kinds of 'refresher courses' may be useful. However, good scientists usually manage to keep abreast of their subject, and even find little difficulty in dealing with the massive literature reporting new work. It has always been the practice in the better institutions for staff to be encouraged to visit other laboratories where advances are

being made, and to attend special courses to learn new methods and techniques.

Instruction in such scientific advances is seldom the concern of the training branches which are now becoming so widespread. They are

**Man management****KENNETH MELLANBY**

mainly involved in promoting the teaching of 'management'. In this, they have been encouraged by reforms in the scientific civil service following the Fulton Report: changes intended to improve efficiency and promotion prospects. Trades unions have also pressed for this type of course, believing that it may enhance their members' prospects, even if

there are doubts as to whether those attending obtain any real benefit.

I have had the opportunity of trying to assess the effects of these management courses on a good many of my colleagues—men and women employed primarily to do research. I have been unable to detect any improvement in their originality or their productivity, or even in their ability to handle their juniors (or their seniors). The courses seem totally useless in promoting scientific research, they waste time which should be spent in the laboratory and they use scarce funds needed for the research itself. I have found this view shared by the directors of many other establishments.

However, the hope is often expressed that, at a suitable stage in their careers (perhaps when their originality is flagging) more scientists will move to senior administrative posts, and that those who have studied management will then be at an advantage. Has anyone ever carried out a controlled experiment, comparing the performance of the 'trained' and the 'untrained'? I have seen no improvement in those who remain in research. Do these management courses really promote effectiveness in scientific administration—or, for that matter, in business or industry? Until these questions can be answered, we should surely be cautious in encouraging these developments. We should certainly not spend money intended for research, except perhaps to do the research needed to evaluate the courses themselves.

# news and views

## Some singular proposals

from P. C. W. Davies

ONE of the more intriguing aspects of the theory of relativity is the prediction that the theory itself will inevitably break down in certain plausible situations. When this happens, space-time is said to develop a singularity. Usually, singularities are associated with intense gravitational fields which grow without limit, such as may occur after the catastrophic implosion of a star to form a black hole. Physicists have always been disturbed about singularities, because if they are taken literally, they imply that there can be regions of the Universe where space-time develops an edge or boundary, at which all of known physics breaks down. Many have recoiled at this possibility, and view the singularity, less catastrophically, merely as an indication that classical relativity ceases to apply under some very extreme conditions. In the alternative tradition singularities are regarded as actual physical entities of some sort, presumably with their own laws and properties. The issue is of literally cosmic importance because it is widely supposed that a singularity lay at the initiation of the big bang *primaeval* phase of the Universe. If this singularity is real, it represents a past temporal extremity of the Universe, which, in somewhat anachronistic terminology, means that there was once a creation. If, on the other hand, the singularity is really just a jargon word for a new region of the physical world about which we are presently ignorant, then the Universe will be infinitely old, with all that implies for thermodynamic paradoxes and time asymmetry.

Often the distinction between these alternative viewpoints is blurred. However, Stephen Hawking of Cambridge University, who is never one to be faint-hearted in facing tough questions squarely, has made some challenging proposals about the nature of singularities, particularly with regard to black holes. In a thought-provoking paper entitled 'Breakdown of predictability in gravitational collapse', (*Phys. Rev. D.*

**14**, 2460; 1977) Hawking argues for the inevitability of singularities and proposes to make a virtue of them by introducing a new physical principle to stand alongside the Heisenberg uncertainty principle. He claims that the breakdown of physics at singularities does not merely signal our ignorance of the correct theory but represents "a fundamental limitation to our ability to predict the future" a limitation which is in addition to the usual quantum uncertainty. This he calls the principle of ignorance.

Hawking discusses in detail a number of situations in which the principle of ignorance can be applied. In all cases there is an intimate connection between singularities and so-called 'hidden surfaces'. General relativity permits the causal structure of spacetime to develop features such as event horizons, beyond which no information is available. In the case of the gravitational collapse of a star the formation of an event horizon leads to the famous thermal evaporation process discovered by Hawking in 1974, and which endows the black hole with a temperature and entropy. The black hole entropy can best be understood as the loss of information behind this horizon. The main part of the paper is concerned with a detailed mathematical treatment of the evaporation process, and confirms the independent work of Parker and Wald that this radiation is truly thermal in the sense that the event horizon appears to emit with equal probability all configurations of particles consistent with the observers' limited knowledge about the black hole (its mass, charge and angular momentum). The precise connection between the horizon and the singularity is a subtle one, and Hawking's argument seems to depend upon an interpretation of the evaporation process in terms of Feynman's idea of anti-particles being particles travelling backwards in time. The evaporation process entails the creation of particle-anti-particle pairs outside the horizon with

one particle falling into the black hole (and thence into the singularity) and the other perhaps escaping to infinity to contribute to the evaporation radiation. Hawking regards this situation as a single particle emanating from the singularity backwards in time, tunnelling to the region outside the black hole, where it scatters off the gravitational field into the future direction, and thereby arrives at infinity. In this way the accepted randomness of the evaporation radiation can be regarded as reflecting a property of the singularity, and is hence a direct consequence of the principle of ignorance. One feature which makes me uneasy about this picture is its dependence on an intuitive, semi-classical idea of particles close to, and inside the black hole, even though the quantum wavelength of the particles is comparable to the size of the black hole itself.

Another situation considered by Hawking, which does not involve an event horizon, concerns the big bang creation of the Universe. Here the hidden surface means any surface sufficiently close to the initial singularity about which we have no previous knowledge. The particles emerging from the singularity will then, according to the principle of ignorance, be random and uncorrelated. This idea is not of course new and has frequently been invoked to account for the observed time asymmetry of the Universe (such as the absence of advanced radiation coming from the big bang). Hawking argues that the characteristics of the cosmic thermal microwave background be explained this way. For example, the high isotropy is beautifully accounted for by noting that an anisotropy can be regarded as the presence of a very large excess of long wavelength gravitons in some modes, which, according to the principle of ignorance, is overwhelmingly improbable.

At a technical level, Hawking points out that the loss of information behind the event horizon of a black hole prevents us from using a pure state to



describe the quantum system. The conventional procedure of introducing an S matrix therefore fails when black holes are present. Instead one has to deal with density matrices. This modification could be profound in quite general circumstances if quantum gravity effects are taken into account, because virtual black holes might form out of high energy virtual photons when the wavelength approaches the Planck length.

With such a concentration of provocative new ideas, this paper is bound to generate a great deal of debate about fundamental physics. In particu-

lar, Hawking's argument for the inevitability of singularities will be challenged by those who have long sought to avoid them. They could be avoided if quantum matter effects can lead to the violation of the so-called energy conditions. Indeed, negative energies and pressures have long been known to be possible in quantum field theory.

The conclusion which Hawking draws is stated with characteristic wit and daring. There is a new level of uncertainty or randomness in physics because "God not only plays dice, He sometimes throws the dice where they cannot be seen". □

## Genetic modification of plant cells: a reappraisal

from Edward C. Cocking

CELL genetics is now one of the most rapidly developing areas of plant biology, stimulated by the considerable improvement over the past decade in our knowledge of plant tissue and cell culture, and in particular the availability of isolated plant protoplasts. Genetic modification as a result of the fusion of isolated protoplasts and the regeneration of somatic hybrid plants has advanced dramatically in the past five years.

One of the more controversial aspects of recent developments in genetic modi-

fication has been the question of the modification of plant cells by exogenous DNA (see for example Ledoux *Nature* **249**, 17; 1974). The general view now is that the present genetic evidence for the modification of plant cells by prokaryotic DNA is circumstantial and weak. Kleinhofs *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **72**, 27481; 1975) concluded that contamination of plant material by bacteria was the likely explanation for what had been interpreted as evidence for the integration of exogenous DNA, as

related to the appearance of "hybrid" bands on centrifugation, since no "hybrid" bands ever turned up provided a stringent sterilisation procedure was used. A re-examination last year by Redei *et al.* (*Proc. Int. Course on Cell Genetics in Higher Plants* (eds Dudits, D. *et al.*) Akadémiai Kiadó Budapest, 1976) of the correction of genetically thiamineless *Arabidopsis* mutants by bacterial DNA is of particular interest. Using supposed DNA-corrected mutants, supplied by L. Ledoux and others, these workers concluded that the 'corrected mutants' were mechanical contaminants either of Langridge's mutant 1018-6, or other mutants, or were wild type. No evidence of DNA-mediated correction in the material examined could be found. So it must now be recognised that even though the work from Ledoux's laboratory has been a great stimulus, on present evidence there are explanations other than genetic modification for the results obtained.

Somewhat similar conclusions can be reached regarding the work of Hess and collaborators. They observed that treatment of a white-flowering pure line of petunia with the DNA of a red-flowering anthocyanin-synthesising pure line results in a genetically stable correction for anthocyanin synthesis in 0.06% of the treated plants. Bianchi and Walet-Foederer (*Acta botan. neerl.* **24**, 1; 1974) have cogently argued that Hess

THE function of the thymus as one of the controlling organs in the ontogeny of immunological responsiveness was defined in the early 1960s. The argument about the manner in which this function is exercised has sputtered ever since. The protagonists of the view that cell outflow from the thymus is largely responsible engendered the concept of the 'T' lymphocyte of thymic origin which is part of our contemporary mythology. The alternative notion, that the thymus functions as an endocrine organ, has led a more chequered life. Its latest form is that the thymus does exert a humoral influence but on lymphocyte populations inside the thymus and perhaps on T cells outside the organ. As J.-F. Bach *et al.* indicate in this issue of *Nature* (page 55) the idea is attractive but the evidence has so far not been compelling.

Bach and his colleagues have concentrated on the isolation and characterisation of a thymus-dependent entity in the serum of pigs. Their material, which they designate *facteur thymique serique* (FTS), they find to be a nonapeptide of determined sequence which

## Serum thymic factor

from A. J. S. Davies

they have now synthesised.

The test system used during the isolation procedure of Bach and his colleagues relied upon the capacity of FTS to influence spleen cells from thymusless mice in the sensitivity of their rosette-forming capacity to inactivation by either azathioprine or anti-theta antiserum. This assay is, as Bach himself says, esoteric and it may or may not reflect the manner in which the same material influences cells *in vitro*. In other studies it has been found that partially purified FTS can induce theta positivity, that it can alter the cytotoxic potential of T cells from thymusless mice, that it can sometimes enhance mitogenic responses of lymphocytes and that it can influence the immunological reactivity of the autoimmune NZB mice. All this evidence suggests that FTS can affect T cells, perhaps by modification of their cell membrane, but is far from the proof of either the existence or identity

of a thymus hormone and Bach correctly refrains from making such a claim.

Bach's approach has been unusual in concentrating on serum as a source of 'thymus hormone'. It will now be possible to determine whether materials similar to FTS can be obtained by squeezing the thymus itself. Also injection of labelled synthetic material will allow its tissue predilections, if any, to emerge. It would be ironic, but not without precedent in the endocrine system, if FTS production was not in the thymus, but only regulated by it.

FTS will doubtless be widely synthesised and may, like transfer factor, be used for a variety of clinical purposes. There are very many factors which either emanate from or are dependent upon the various components of the lymphoid system. Their disentanglement will rest upon the extent to which their characterisation can be achieved. Bach, in presenting a sequence for a small readily-synthesised biologically active peptide, will certainly engender much fluttering in the lymphocyte doves. The effects of this disturbance could be considerable. □

did not take the existing differentiation of the shoot apex into account, when he attempted to interpret his experimental results, and that other explanations are possible for the effects observed (for a detailed critical discussion see Hess in *Plant Cell, Tissue and Organ Culture* 506 (eds Reinert, K. J. & Bajaj, Y. P. S.) Springer-Verlag, Berlin-Heidelberg-New York, 1977).

The recent report by Zryd (*Abstract 191, 9th Int. Conference on Plant Growth Substances*, 1976 (ed. Pilet, P. E.) that sycamore cell suspensions plated at low cell density at certain stages of the growth cycle are able to utilise lactose as a sole carbon source is now stimulating a reappraisal of other work using transducing phage to introduce selected bacterial genes into plant cells. Using  $\lambda$ plac5-treated sycamore cell suspensions Johnson *et al.* (*Nature new biol.* 244, 105; 1973) concluded that the *lac* genes were expressed, leading to the formation of enzymes which confer the capacity to assimilate lactose. They stated that their untreated sycamore suspension cultures were unable to use lactose as a sole carbon source.

Somewhat similar experiments using tomato callus have been carried out by Doy *et al.* (*Biochemistry of Gene Expression in Higher Organisms* (eds Pollak, J. I. & Wilson Lee, J.) Reidel, New York, 1972). These results were particularly persuasive, as various bacteriophage controls were used. Inoculation with  $\lambda$ gal<sup>+</sup> enabled callus to grow slowly on 2% galactose medium for up to 15 weeks, whereas untreated cells and the controls ( $\lambda$ gal<sup>-</sup> with an amber nonsense mutation in the *gal* operon) died within 3 weeks. In the light of Zryd's results these experiments should be reassessed; larger populations of cells need to be screened to determine whether tomato, and sycamore, can grow on galactose, and lactose, as a sole carbon source in certain cultural conditions.

Such alternative explanations for the phenomena observed, which have nothing to do with genetic modification, should be rigorously explored. Although it has been claimed that in tomato callus treated with  $\Phi$ 80plac<sup>+</sup>, specifically *E. coli*  $\beta$ -galactosidase is synthesised, no such positive indication was obtained by Johnson *et al.* It is claimed that the biochemical-immunological test of specific protection at 60 °C is regarded as definitive at the molecular level for bacterial  $\beta$ -galactosidase, but this statement also needs critical appraisal, particularly since it is known that plant  $\beta$ -galactosidases are present in sycamore cells (Keegstra & Albersheim *Plant Physiol.* 45, 675; 1970), and probably also in tomato callus

A more promising avenue of approach is the possible use of bacterial plasmids rather than transducing phage for the transfer of prokaryotic DNA to plants. The limitations of the present work on genetic modification as a result of DNA uptake are now evident, but there does still exist the possibility of achieving the desired goal. It is now clear that crown-gall disease caused by *Agrobacterium tumefaciens* is the first example of a natural system in which genetic elements of bacterial origin (the Ti plasmids) play a part in the regulation of gene expression in eukaryotic cells (Schell *et al.* in *Molecular Biology of Plants* (ed. Rubenstein, I.) Academic, New York, 1977). This raises the possibility of using the Ti plasmids for introducing specific bacterial genes into plants.

The most interesting applications would be the integration of the nitrogen-fixing genes (from *Rhizobium* and other bacteria) into crop plants. The *nif* genes of *Klebsiella* have recently been translocated onto the RP4 factor (Dixon *et al.* *Nature* 260, 268; 1976). Schell and his colleagues have suggested that this RP4-*nif* factor could be integrated into the Ti plasmid, which could then be assessed for its ability to make plant cells nitrogen fixing. □

## Sexual transmission of hepatitis B

from Arie J. Zuckerman

SENSITIVE techniques for detecting hepatitis B markers have over the past few years revolutionised epidemiological concepts of this infection. The estimated 120 million carriers worldwide cannot be accounted for simply by transmission through infected blood and blood products and the question of whether an apparently healthy carrier can frequently transmit the infectious agent by any other means is now of crucial importance.

Hepatitis B surface antigen, a virus marker, has been found repeatedly in human saliva, menstrual and vaginal discharges, seminal fluid and milk. It has also been reported but not confirmed in bile, sweat and tears. So it is not surprising that contact-associated hepatitis B is apparently of major epidemiological significance, and various aspects of the growing evidence for intimate transmission of the virus were discussed at a postgraduate symposium on hepatitis B held as part of the American Association for Study of Liver Disease meeting last November.

Studies on transmission by intimate contact have however produced varied and sometimes conflicting results. In

particular studies on possible transmission by sexual contact have produced ambiguous results. Since the surface antigen has been detected in menstrual blood, vaginal secretions and semen, it is possible that the virus could cross mucosal surfaces during intercourse.

The first indications of possible sexual transmission came some 50 years ago when the husbands and wives of known hepatitis B carriers developed acute hepatitis. Recent studies on the sexual transmission of hepatitis have yielded a wealth of good circumstantial evidence, but also conflicting and sometimes ambiguous information. For example, Mosley (*Am. J. med. sci.* 270, 253; 1975) reported that as many as 18% of the spouses of patients with acute hepatitis B, but none of their other close family, contracted the infection. Heathcote and Sherlock (*Lancet*, i, 1468; 1973) also concluded that sexual contact or close domestic contact seemed to be the most likely source of hepatitis B infection in 40% of 67 patients studied. But a similar frequency of hepatitis B surface antigen was found in registered prostitutes in Athens and in control pregnant women of similar age and socioeconomic status; in prostitutes in Germany and mentally retarded patients in institutions, and among prostitutes and nuns in Columbia.

Nevertheless, several studies carried out in London by a team from the Middlesex Hospital seem to substantiate the possibility of sexual transmission of hepatitis B infection. A high prevalence of surface antigen and surface antibody (the latter providing evidence of infection with hepatitis B virus) has been found in patients attending venereal disease clinics. Promiscuity and in particular homosexual promiscuity emerged as factors of special importance. Thus in one of the studies, homosexuals were found to carry the surface antibody seven times more frequently than their male heterosexual counterparts. In another survey antigen was found ten times more frequently in patients attending a venereal disease clinic in London than in volunteer blood donors (bearing in mind, of course, that these groups are not strictly comparable). Again it was noted that the highest incidence of hepatitis B surface antigen was among male homosexuals.

In the most detailed study to date, of over two thousand persons in New York, Szmuness *et al.* (*Annals intern. Med.* 83, 489; 1975) evaluated two separate aspects of the role of sexual behaviour in the spread of hepatitis B: first, the possibility that this infection can be transmitted by vaginal intercourse and therefore that hepatitis B may be regarded as a venereal disease;



and second, the indirect impact of certain sexual practices on the spread of hepatitis B virus in various population groups. Serological evidence of infection was sought by very sensitive laboratory techniques. A significant excess of hepatitis B infection was found in two promiscuous groups: patients with venereal diseases and their unrelated sexual contacts (15–18%); and male, but not female, homosexuals (37–51%) (volunteers contacted through homosexual organisations). The prevalence of antigen among male homosexuals was 13 times higher than in the controls, and surface antibody was found 4–5 times more frequently than in the controls. 26 to 28 per cent of spouses of asymptomatic antigen carriers were infected; an infection rate 2–3 times greater than that among spouses of the controls.

Serological evidence of infection with hepatitis B virus was found more frequently in individuals who had a higher than average probability of exposure to potentially infective partners or whose sexual behaviour made such exposure more likely. Large numbers of sexual partners, long duration of homosexuality and predominantly rectal intercourse all contributed to a higher frequency of infection. No antigen carriers were detected in the admittedly small lesbian sample, although antibody was found at the same frequency as in heterosexual women in the general population. This low prevalence may be related to the comparative numbers of sexual partners; in the homosexual sample surveyed the mean number of partners during the preceding six months was 1.8 for women compared with 20 for men.

An unexpected finding reported by Szmunn and associates (*op. cit.*) was the high prevalence of hepatitis B in homosexuals whose sexual behaviour included rectal intercourse, and a relatively low prevalence in homosexuals with practices beyond the bounds of the anogenital system. The explanation for this difference may lie in the reported high incidence of recurrent genital and perianal skin and mucosal sores in the former group.

However, it is extraordinarily difficult to prove by such a cross-sectional retrospective study that hepatitis B can be transmitted venereally in addition to the many other potential modes of transmission. Not only is it difficult to collect reliable information on sexual behaviour, but spouses and highly promiscuous groups differ from each other in many respects, posing problems which cannot be overcome by statistical methods. Nevertheless, several studies have now confirmed that in certain groups intimate contact with large numbers of sexual partners

and extravaginal intercourse may be responsible for a substantial proportion of clinically apparent or inapparent infection with hepatitis B virus. But it still could not be proven that type B hepatitis can be transmitted by vaginal intercourse. H. O. Conn, in a paper delivered at the American Association for Study of Liver Disease meeting,

pointed out that "it must be borne in mind that people who sleep together may do other things as well. They may share eating utensils, razors, toothbrushes, skin abrasions, needles, lovers and bed bugs; and any of these or others could permit the intimate, but non-venereal transmission of viral hepatitis". □

## Super-rotating atmosphere of Venus

from Garry E. Hunt

THE atmosphere of Venus is huge, composed mainly of carbon dioxide with ubiquitous clouds obscuring the surface which is characterised by temperatures of about 730 K and pressures of nearly 100 atmospheres. Centuries of visual observation provided little more than frustration to the early astronomers with the regular sight of a yellow, featureless cloud deck, which extends to nearly 100 km above the planetary surface, in complete contrast to Earth which averages about 50% cloud cover. But planetary observations in ultra-violet light have provided the most exciting and unexpected result which still remains today as a major unsolved problem in Venusian meteorology. The upper atmosphere in the neighbourhood of the cloud tops super-rotates. Features in this region move in a retrograde manner with a rotational period of only 4 d which is incredibly short when compared with the planet's own solid-body retrograde rotation of 243 d. So Venus possesses the largest ratio between the atmospheric and planetary rotation rates of any body in our Solar System.

Initial evidence of this atmospheric rotation came from the apparent motion of dark features seen in ultra-violet photographs, characterised by Y and  $\psi$  shapes centred on the equator (Boyer & Camichel *Annls Astrophys.* **24**, 531; 1961). But is it really a mass motion that is observed? A wave phenomenon propagating around the planet with a phase velocity corresponding to a 4-d period could produce a similar effect. Although Young (*Icarus* **24**, 1; 1975) considered the super-rotation an illusion, a travelling wave-like disturbance driven by the ultra-violet albedo differences, spectroscopic measurements of winds by Traub and Carleton (*J. atmos. Sci.* **32**, 1045; 1975) and the results of the Venera 8 Doppler drift experiment (Marov *et al. J. atmos. Sci.* **30**, 1210; 1973) provide strong evidence that the visible parts of the Venusian atmosphere really are moving rapidly with respect to the planetary surface. The measured wind

velocity is large enough to account for most, if not all, of the 100 m s<sup>-1</sup> apparent equatorial motion seen in the ultra-violet markings

But how persistent are the observed features? According to Boyer and Guerin (*Icarus* **11**, 338; 1966) the Y feature persists for decades at essentially the same longitude in a coordinate system rotating with a period of 4.067 d. On the other hand, Beebe (*Icarus* **17**, 602; 1972) and Scott and Reese (*Icarus* **17**, 589; 1972) thought that owing to the selective nature of the ground-based observations, the features may actually appear at random longitudes and dissipate after 8–16 d. The high resolution images taken during the Mariner 10 flyby are valuable observations in the study of the lifetime of these features, since Dollfus (*J. atmos. Sci.* **32**, 1060; 1975) has shown them to be representative of the more extensive time series of Earth-based measurements. Belton *et al.* (*J. atmos. Sci.* **33**, 1363; 1976) found that the large scale markings (~1,000 km) situated between  $\pm 45^\circ$  latitude have lifetimes greater than 4 d and more with an apparent angular motion at the equator. Smaller scale markings (100–500 km) were found to have lifetimes between 1.5 and 4 d. These observations suggested to Belton *et al.* that the Y and polar ring features seen in the images are both visible manifestations of propagating waves.

But we are still faced with the fundamental question—what is the mechanism(s) capable of generating these large zonal winds? The overhead motion of the Sun relative to an observer fixed on the surface is prograde with a speed of about 4 m s<sup>-1</sup>. Any theory must then account for the retrograde flow of the order of 100 m s<sup>-1</sup>. Gold and Soter (*Icarus* **14**, 16; 1971) proposed that the upper atmosphere circulation is a result of a solar torque on a thermal semidiurnal tide. Winds of the observed magnitude could be produced in this way only if the momentum transport were molecular. If, however, turbulent eddies provide the



main source of viscous friction, then the mechanism is incapable by several orders of magnitude of accounting for the observed winds.

Schubert and Whitehead (*Science* **163**, 71; 1969) suggested that the 4-d circulation is driven by the overhead motion of the Sun relative to the planet's surface. This is known as the "moving flame mechanism". The solar energy is absorbed in the visible cloud tops situated in the upper troposphere and its effects conveyed to higher levels by various transport mechanisms. Because these mechanisms do not act simultaneously the horizontal movement of the source of heat produces a tilt in the vertical pattern of convection. Associated with the tilt are the Reynolds Stresses which, by transporting horizontal momentum upwards or downwards produce and maintain an altitude-dependent streaming motion. This viscous action transfers horizontal momentum vertically at an equal rate in the opposite direction.

General circulation models, such as those which have been applied to the Earth's atmosphere suggest a numerical approach to the study of atmospheric dynamics. Their first application to the problems of the upper atmosphere of Venus was not very successful (see for example Kalnay de Rivas *J. Atmos. Sci.* **30**, 763; 1973) producing maximum winds of only  $8\text{--}10\text{ m s}^{-1}$ , which is an order of magnitude less than those observed. Young and Pollack (*Science* (in the press, 1977)) now report a more satisfactory study producing the observed retrograde mean zonal wind of  $90\text{--}100\text{ m s}^{-1}$  corresponding to a 4-d rotational period. At an altitude of 60 km their model results show meridional velocities of  $10\text{--}20\text{ m s}^{-1}$  at mid and high latitudes, a 12 K average equator-pole temperature difference and 3 K day-night temperature difference with the night side hotter. These characteristics agree reasonably well with the observations. Young and Pollack suggest that the large zonal winds in the Venusian atmosphere are the result of a non-linear instability, although the rotation of the planet and the motion of the Sun relative to the surface (moving flame principle) play important parts in determining the direction of the mean winds generated from an initially stationary state. Thompson (*J. Atmos. Sci.* **27**, 1107; 1970) first indicated the importance of this instability mechanism which results from the perturbation to a steady convective pattern caused by the superimposing of a vertical shear on this original flow. In the Venusian atmosphere, the relatively large shear is the result of the vertical distribution of solar energy, which is

mainly deposited above the 40 km altitude.

The latitude dependence of the mean flow produced by the Young and Pollack model corresponds to a constant angular velocity at a given vertical level. This disagrees with the Mariner 10 observations, which show a more complicated picture of an increase in angular velocity from the equator to a latitude of approximately  $50^\circ$  (Murray *et al.* *Science* **183**, 307; 1974), where the rotational period may be as short as 2 d. Spiral streaks, which may be interpreted as 'jet streams' show in the Mariner 10 images the flow towards the polar vortices where the kinetic energy of the motions is eventually dissipated.

The return flow of atmospheric gas may occur at deeper levels in the atmosphere in order to maintain the planetary angular momentum balance. We do not know the vertical extent of these motions, whether the stratospheric rotation is a separate overturning cell or whether it is linked somehow to a deep stirring of the huge Venusian atmosphere. Sidi (*Icarus* **28**, 359; 1976)

found that a long and narrow striplike cloud formation located within the equatorial region and parallel to the equator appeared to move differently from other clouds in the same area. This suggests that inhomogeneities in the upper atmosphere may exist at two altitude levels at least.

Why is meteorology of the upper atmosphere of Venus so different from that of Earth? The super-rotation requires an efficient high altitude thermal forcing, but for the Earth, the largest amount of available radiative energy is that absorbed at the surface. Could other planetary atmospheres exhibit this excess rotation? Leovy (*J. Atmos. Sci.* **30**, 1218; 1973) suggests that any atmosphere where heating tends to produce an equatorial thermal bulge, but which cannot develop instabilities because of slow rotation or large damping, must develop an excess rotation in the same sense as the planetary rotation at a high level. The super-rotation of the Earth's thermosphere reported by King-Hele *et al.* (*Planet. Space Sci.* **18**, 1433; 1970) may be another example. □

## Muscle diseases at Birmingham

from E. J. Thompson

The Seventh Symposium on current research in muscular dystrophy organised by the Muscular Dystrophy Group of Great Britain was held at the University of Birmingham on 5-7 January 1977. It consisted of papers invited from workers supported directly by grants from the Muscular Dystrophy Group, and was organised by Professor J. N. Walton, Chairman of the Muscular Dystrophy Group of Great Britain and Professor Sir Andrew Huxley, Chairman of the Medical Research Committee.

At the meeting various aspects of the development of innervation and muscle biochemistry as well as metabolic, immunological and genetic aspects of human muscle diseases were discussed.

One of the basic questions addressed was—how does nerve leave its molecular footprint on muscle? During normal development, muscle is highly and uniformly sensitive to acetylcholine over its entire surface. However, after innervation, sensitivity is decreased everywhere except at the site of nerve contact. With denervation, the muscle reverts to its less differentiated state, yet the original site of nerve contact remains more sensitive

than the surrounding membrane. C. R. Slater (Newcastle General Hospital) described an elegant model which allows systematic study of this phenomenon (work done in collaboration with T. Lomo (University of Oslo)). This involves the implant of a second nerve which requires only 3 days contact with the muscle before it leaves the characteristic cluster of acetylcholine receptors in higher density than the contiguous membrane. One can then ask what is the nature of the molecular cement which maintains the peculiar quaternary structure of these receptor proteins?

G. Vrbova (University College London) compared the difference in the chick embryo pattern of innervation between fast and slow muscle (that is, single rather than multiple synapses per fibre) respectively. She noted that fast muscle was less sensitive to acetylcholine generally and has synapses with a larger space constant. It is thus possible that during normal development the nerves which are destined to induce fibres to become fast muscles exert their particular influence by depolarising a larger area along each muscle fibre.

The increasing use of tissue culture to investigate myogenic cell behaviour in defined conditions has necessitated single cell assays for specific gene products. One such probe has been directed against messenger RNA. K. W. Jones (Institute of Animal Genetics, Edinburgh) has isolated mRNA from

heavy polysomes which synthesise two heavy chain myosin molecules. By making a labelled copy of complementary DNA, one can then show selective binding to fixed myotubes and to the cytoplasm between fusing myoblasts. There was no binding to fibroblasts in the same culture. Another specific probe is antibody directed against the individual polymorphic forms of myofibrillar proteins. S. V. Perry (University of Birmingham) has shown that antibody to  $\alpha$ -tropomyosin as well as that against troponin T will detect individual fibres in a mixed muscle. Further work on  $\beta$ -tropomyosin as well as troponin I should be forthcoming.

In the discussions on human muscle disease J. A. Simpson (Southern General Hospital, Glasgow) reviewed the evidence that myasthenia gravis is an autoimmune disease. Although there have been some difficulties in transferring the complete myasthenic effect into animals, J. Newsom Davis (Institute of Neurology, London) and R. Miledi (University College London) have found that neuromuscular transmission can be blocked *in vitro* by sera from myasthenic patients. However, Newsom Davis also stated that the level of antibody against the acetylcholine receptor may not closely parallel the clinical status of the patients.

There are several good reasons for considering the role of the cell surface in these diseases. P. D. Smith (Charing Cross Hospital, London) reported that patients with polymyositis had circulating lymphocytes with an increased affinity for intact cultured myotubes and a provocative single patient with myasthenia gravis had a five-fold increase in preferential attachment to the muscle. W. J. K. Cumming (Newcastle General Hospital) found that histocompatibility group BW14 was increased ( $P < 0.001$ ) in a group of patients with polymyositis. R. Yasin (Institute of Neurology, London) showed an abnormal morphology and behaviour (clustered cells) in cultured muscle biopsies from Duchenne patients.

There are certain reservations as to how closely the mouse model of dystrophy parallels the human situation, but there are, nevertheless, several useful parameters which can be further pursued with this system. M. R. West (Newcastle General Hospital) found that dystrophic mouse, (*dy* 1) muscle appeared to reach a lower level of differentiation than normal muscle in a number of different culture conditions.

The pendulum is clearly swinging back from the neurogenic to the myogenic hypothesis of muscular dystrophy. Recent findings now show abnormalities in the dystrophic muscle

without exogenous nerve. The most exciting implications derive from abnormalities on the cell surface which could be linked to both intrinsic biochemical abnormalities as well as a general defect in cell-cell signalling. The question remains as to why there should be pseudohypertrophy of the muscle, that is, proliferation of abnormal amounts of interstitial cells. □

## Vitamin D at Asilomar

from Alastair Hay

The Third Workshop on Vitamin D was held in the Asilomar Conference Grounds California, from 9–13 January 1977. The Proceedings of the Workshop, which was organised by A. W. Norman, will be published in full by Walter de Gruyter Co., New York.

THE exact mechanism of action of vitamin D ( $D_2$  and  $D_3$ ) still remains unresolved nearly 60 years after Mellanby reported the presence of an antirachitic factor in cod-liver oil; considerable progress has, however, been made over the past 10 years.

It is well known that ultraviolet irradiation of 7-dehydrocholesterol in the skin will ultimately produce vitamin  $D_3$  (cholecalciferol), but the *in vivo* mechanism of production is not well understood. M. F. Holick (Massachusetts General Hospital, Boston) has now shown that the initial product isolated from the skin of rats given no treatment other than ultraviolet irradiation is previtamin  $D_3$ , thus demonstrating for the first time that *in vivo* the pathway is at least 7-dehydrocholesterol  $\rightarrow$  previtamin  $D_3 \rightarrow$  vitamin  $D_3$ .

Until a few years ago there was no reliable assay for the measurement of the dihydroxy metabolites of vitamin  $D_3$  at the physiological level. Now, however, there are two alternatives for the active hormonal form of vitamin  $D_3$ , 1,25-dihydroxycholecalciferol ( $1,25-(OH)_2D_3$ ), the assays of Brumbaugh *et al.* (*Science* **183**, 1089; 1973) and Eisman *et al.* (*Science* **193**, 1021; 1976). There may eventually be a third possibility. This was proposed by G. Jones (Hospital for Sick Children, Toronto), who demonstrated that using ultraviolet detectors of sufficient sensitivity, high pressure liquid chromatography (HPLC) can be used to routinely assay vitamin D, 25-hydroxyvitamin-D ( $25(OH)D$ ), and  $1,25(OH)_2D$ . Since there is no specific assay for vitamin D itself, and competitive protein binding

assays only measure the concentration of an individual metabolite, there would seem to be definite advantages to HPLC for these routine estimations.

Levels of the metabolite 24,25-dihydroxycholecalciferol ( $24,25(OH)_2D_3$ ) found in various studies still prove difficult to interpret. In human volunteers with levels of plasma  $25(OH)D$  in the range 6.0–36.0 ng ml<sup>-1</sup>, C. M. Taylor (University of Manchester) reported circulating levels of  $24,25(OH)_2D_3$  within the range 0.3–3.6 ng ml<sup>-1</sup>, and the absence of this dihydroxy metabolite in anephric patients. J. G. Haddad (Jewish Hospital of St Louis, Missouri) however, reported circulating  $24,25(OH)_2D$  levels 2–3 times those of Taylor's, and in addition, normal levels of the metabolite in sera from anephric patients. Since anephric patients are missing the kidney enzyme vitamin D 24-hydroxylase, Haddad suggests that significant extrarenal 24-hydroxylase was present in his subjects.

Additional evidence for an extrarenal 24-hydroxylase was provided by H. F. DeLuca (University of Wisconsin); his laboratory has observed the conversion of  $1,25(OH)_2D_3$  to  $1,24R,25$ -trihydroxycholecalciferol ( $1,24R,25(OH)_3D_3$ ) *in vivo* in nephrectomised rats and chicks. There is still some scepticism concerning the physiological importance of the trihydroxy metabolite but DeLuca reported that it is present in sufficient quantity *in vivo* to be regarded as significant.

The active form  $1,25(OH)_2D_3$  is rapidly accumulated in target tissues. In the gut, for example, it regulates the transcription of the calcium binding protein (CaBP) gene. R. H. Wasserman (Cornell University, Ithaca) reported that in chick intestine in steady-state conditions—such as adaptation to egg laying or calcium or phosphate deficiency—his previous data showed good correlation between CaBP concentrations and calcium absorption. More recent data, however, suggest that another 'vitamin D responsive factor' in addition to CaBP is required for vitamin-D stimulated calcium absorption.

Other 'vitamin D responsive factor(s)' have been observed in the brush border membrane of chick intestine. D. E. M. Lawson (Dunn Nutrition Laboratory, Cambridge) has observed at least two membrane proteins which are synthesised more rapidly than CaBP in response to  $1,25(OH)_2D_3$ . And H. Rasmussen (Yale University School of Medicine, Connecticut) reported changes in the fatty acid composition of the membrane phosphatidylcholine (PC) fraction in membranes isolated from vitamin-D deficient chicks treated with  $1,25(OH)_2D_3$  compared with vitamin-D deficient controls. The changes observed in the PC fraction of the



treated chicks—associated with an increased ability of the membrane to take up calcium—were an increase in the  $\omega:6$  fatty acids linoleic and arachidonic, and a decrease in at least one of the  $\omega:3$  series, docosahexaenoic acid.

The functions of the two dihydroxy metabolites  $24,25(\text{OH})_2\text{D}_3$  and  $25,26(\text{OH})_2\text{D}_3$  are still far from being resolved. J. A. Kanis (Nuffield Orthopaedic Centre, Oxford) suggested that  $24,25(\text{OH})_2\text{D}_3$  may act directly on bone to increase mineralisation, on the basis of the observation that  $1\mu\text{g d}^{-1}$  of  $24,25(\text{OH})_2\text{D}_3$  (R+S epimers) administered to an anephric patient with osteomalacia, increased intestinal calcium absorption and reduced plasma alkaline phosphatase, without causing an increase in plasma or urine calcium. These effects were not observed by A. M. Pierides (University of Newcastle upon Tyne) however, in patients receiving as much as  $16\mu\text{g d}^{-1}$  of  $24,25(\text{OH})_2\text{D}_3$ .

The most ignored dihydroxy metabolite of vitamin D is certainly  $25,26(\text{OH})_2\text{D}_3$ . Very little is known about

its properties and its *in vivo* origin is completely unknown. That it is a significant metabolite in man at least, was clearly demonstrated by E. B. Mawer (University of Manchester). In vitamin D replete individuals the serum concentrations of  $25,26(\text{OH})_2\text{D}_3$  and  $24,25(\text{OH})_2\text{D}_3$  are similar. Whereas the production of  $1,25(\text{OH})_2\text{D}_3$  and  $24,25(\text{OH})_2\text{D}_3$  are governed by parameters such as the state of vitamin D nutrition and serum calcium concentration, no such controls are evident for  $25,26(\text{OH})_2\text{D}_3$  synthesis.

The papers referred to were only a few of the highlights of this workshop. But they do indicate that the next decade should be fruitful. By the 1980s the functions and mechanisms of action of all the vitamin D metabolites should be known, and the importance of  $1,25(\text{OH})_2\text{D}_3$  in phosphate regulation should be determined. The answers to these questions should provide reliable therapeutic procedures for the treatment of clinical disorders such as the renal osteodystrophies and postmenopausal osteoporosis. □

tion reactions of triplet methylene with hydrogen and methane studied in the same way. For the methane abstraction



the work implies that the reaction should not occur to a significant degree at room temperature contrary to the predictions of Dewar based on his semi-empirical MINDO calculations.

Although it is still early to be sure that the minicomputer will lead to a welcome change of life for quantum chemists it is striking just how wide is the range of problems tackled to date with the relatively cheap form of computer. Not only have quantum mechanical calculations been performed, but semiclassical computations, such as atomic scattering from solid surfaces, have also been studied in this way; an example being the scattering of helium and neon from tungsten, the work of Miller and his colleagues (*J. chem. Phys.* **64**, 45; 1976).

If quantum chemistry can be made inexpensive enough for chemists in experimental research laboratories to run their own calculations then the day may at last come when experimentalists can run their own calculations routinely. This will save the literature being over-burdened with details of computations which may be directly interesting only to a small audience, and will enable practical chemists, such as medicinal chemists, to reap the benefit of useful calculations without needing to persuade a theoretical group to take on their work. □

## Minicomputers for quantum chemists

from Graham Richards

QUANTUM chemists have always been major users of the most powerful computing facilities. Indeed, in recent years, the advances in the subject have followed the introduction of each new generation of machines and the capability of doing the best work has, to a large extent, been limited to those with access to major computing centres. As a result the subject has been dominated by a small number of groups and not used as a tool by more applied chemists to study or clarify their own research problems. The rapidly falling cost and increasing power of minicomputers should lead to a radical change if it were possible to perform the massive *ab initio* quantum mechanical calculations on minicomputers rather than giant number-crunchers. A pilot study which succeeds in getting the quart into a pint pot and studying genuinely interesting problems has been conducted in the Chemistry Department in Berkeley.

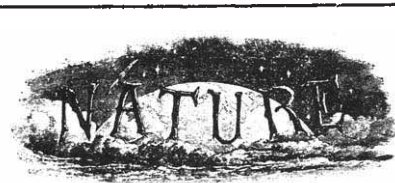
The question "are minicomputers suitable for large scale scientific computation?" was answered in the affirmative by Schaefer in a paper presented at the IEEE Computer Society Conference held in Washington in September 1975 and in the proceedings of that meeting the relative capabilities and suitability of a number of minicomputers are compared. Since that date memory prices have fallen and new developments such as high speed array

processors make the use of the minicomputer a very attractive alternative to the use of expensive large machines.

Calculations may take of the order of 30 to 40 times as long on a minicomputer as they do on a large scientific machine. However, since the calculations may be run overnight and the machine can be operated by graduate students or research personnel rather than professional operators, it may be much cheaper to work in this way.

The molecular structure calculations which have been attempted using a minicomputer have not been limited to the study of simple electronic ground states of closed-shell molecules. The Berkeley group has studied the excited electronic states of both glyoxal and ketene (*J. Am. chem. Soc.* **98**, 401, 2689; 1976). For each of the molecules, some 20 excited states are included and details are given of geometries as well as excitation energies.

The same minicomputer provided the means of studying some fundamental chemical reactions with *ab initio* molecular orbital calculations. Two reactions involving methylene have formed the subject of articles in the *Journal of the American Chemical Society* (**98**, 1653, 3073; 1976). For the least-motion insertion reaction of methylene and hydrogen to yield methane the transition state geometry and activation energy were calculated and the abstrac-



### A hundred years ago

THE total expenditure on the new building at South Kensington for the reception of the Natural History Collections now in the British Museum is stated in the new Civil Service Estimates to have been 206,472l. up to September 30 last. A further sum of 36,650l. is required to carry on the works up to the end of the present financial year. This amount has been already voted. The proposed vote for the present financial year 1877-78 is 70,000l., leaving the amount of 1,878l. necessary to complete the building, the total estimate having been 395,000l. We may remark that it is not only in this country that a new Museum of Natural History is in progress. Both at Paris and at Berlin the present buildings for the National Museum are found to be too small, and large sums are to be appropriated to their reconstruction.

From *Nature* **15**, March 1, 382; 1877.

# review article

## The continental drift debate

F. J. Vine\*

*The controversy within the earth sciences over the hypothesis of continental drift has been unusually protracted. Here Professor Vine reviews the history of the debate as recorded in the pages of Nature.*

THE name most commonly and correctly associated with the hypothesis of continental drift is that of the German meteorologist Alfred Wegener (1880–1930). Wegener's first article on the subject appeared in 1912 and the first edition of his book *Die Entstehung der Kontinente und Ozeane* (The Origin of Continents and Oceans) in 1915. Presumably because of the first World War and the lack of an English translation, a review of Wegener's book did not appear in *Nature* until 1922<sup>1</sup> by which time a second edition was available (published in 1920). It seems probable that this together with Wegener's article in *Discovery* in 1922<sup>2</sup>, constituted the first widespread exposure of the "Wegener hypothesis" to the English-speaking world and it prompted a great deal of discussion at scientific meetings and correspondence in *Nature*.

It is perhaps instructive to ask why it was that geologists had paid little attention to the concept of continental drift before the publication of Wegener's book, for Wegener was certainly not the first to suggest that the continents were once together and subsequently drifted apart. Several contributors to *Nature* in the 1920s were at pains to point out that Wegener was anticipated by, among others, Oswald Fisher (1882), W. H. Pickering (1907), F. B. Taylor (1910) and H. A. Baker (1911); and A. A. Robb in 1930 (ref. 3) unearthed and reproduced the reconstruction of the continents around the Atlantic published by Antonio Snider in 1858. A particularly concise and authoritative account of the philosophical development of the concept before 1900 appeared in *Nature* in 1970<sup>1</sup>. It was written by N. A. Rupke who identified, I think, the main reason why geologists did not consider drift before the publication of Wegener's ideas. All the earlier advocates had associated drift with a catastrophic event. Even Taylor, who, quite reasonably, suggested that drift might explain the formation of the Atlantic and Indian oceans and the young fold mountain belts, invoked the origin of the Moon out of the Pacific in the mid-Cretaceous as the ultimate cause. Although the earliest geologists were well-versed in biblical catastrophism, the concept of 'uniformitarianism' or 'actualism', first advanced by James Hutton and Sir Charles Lyell in the early nineteenth century, gradually replaced this earlier philosophy so that by the turn of the century the invocation of any sort of catastrophic event to explain geological phenomena was something of an anathema to all right-

minded geologists. Surprisingly perhaps, Wegener's hypothesis was the first to formulate the concept of drift within the framework of 'uniformitarianism', that is, by processes operating today which have operated slowly but inexorably throughout time.

In addition Wegener went further than anyone before in attempting to document the geological and geophysical evidence for drift, and to provide, albeit rather unsuccessfully, uniformitarian mechanisms whereby drift might be taking place. Wegener was attacked on all three counts, on the basis of the geological and geodetic evidence he invoked, and the mechanisms he suggested. The debate dragged on inconclusively for 30 yr until even the perspicacious Arthur Holmes was driven to write, in 1953<sup>3</sup> "I should confess that, despite appearances to the contrary, I have never succeeded in freeing myself from a nagging prejudice against continental drift: in my geological bones, so to speak, I feel the hypothesis to be a fantastic one". However, the end was in sight: the advent of new and independent evidence suggestive of drift, from palaeomagnetic studies, resuscitated the idea in the late fifties and early sixties, and subsequently the post-war investment in marine geology and geophysics paid off in the form of providing compelling evidence for sea-floor spreading and hence continental drift. By the late 1960s the vast majority of geologists and geophysicists were convinced that continental drift was a reality; it was as if "the dream of a great poet" (Termier's words in 1926) had come true.

The reviewers of the various editions of Wegener's book which appeared in the 1920s seem to have suffered from a mixture of shock, incredulity and even some initial enthusiasm on reading Wegener's heretical ideas. The reviewer of the second edition<sup>1</sup> refrains from passing judgement, but concludes that "The revolution in thought, if the theory is substantiated, may be expected to resemble the change in astronomical ideas at the time of Copernicus". It is interesting to note that J. Tuzo Wilson made precisely the same analogy 45 years later following the essential confirmation of Wegener's hypothesis<sup>4</sup>. Grenville Cole<sup>5</sup> in a somewhat flowery but careful review of the third edition of Wegener's book could not decide whether to be serious or frivolous in discussing it. On the whole he took it seriously, if only as a challenge, and in so doing made some very interesting points. Gregory's review of the English translation of the third edition by J. G. A. Skerl also makes interesting reading. Gregory is obviously stimulated by the boldness of

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Wegener's ideas and maintains that they were being favourably received by geologists in general at that time, an impression which is not corroborated, however, on reading other items of this period. Gregory is preoccupied with a possible connection between Asia and North America across the Pacific which he deduces on palaeontological grounds. Although he finds this inconsistent with Wegener's theory he finally admits that "The original theory has undergone so many modifications and its author is so delightfully open-minded, that it may be changed to agree with a trans-Pacific land connexion". Indeed more than forty years later J. Tuzo Wilson made just such an accommodation by suggesting that a fragment of southeast Asia (Cascadia) was rafted across the Pacific and accreted on to the western margin of North America<sup>6</sup>.

An early convert to Wegener's ideas, J. W. Evans, writing in 1923<sup>7</sup>, made some rather pertinent observations on continental margins, predicted largely submerged microcontinents and emphasised the futility of fitting coastlines rather than shelf edges in making continental reconstructions. Such a geological *faux pas* was committed as recently as 1962 by Sir Harold Jeffreys<sup>8</sup>. In this letter Jeffreys maintained, as he had done previously, "that Wegener's alleged fit of South America into Africa is a misfit by about 15°. This is obvious on inspection of a globe". Cambridge legend has it that it was this assertion which prompted Sir Edward Bullard to make his well-known and much reproduced 'computer fit' of the shelf edges bordering the South Atlantic<sup>9</sup>. Bullard's fit was near perfect; Sir Harold, of course, had been referring to the coastlines. Bullard's fit is in fact virtually identical to that obtained nearly 10 yr earlier by S. W. Carey using an analogue technique<sup>10</sup>.

Carey, Professor of Geology at Hobart, Tasmania, is one of a long line of southern hemisphere geologists who championed drift through thick and thin, largely on the basis of the geological record of the southern continents plus India which suggests that they formed a single super-continent—Gondwanaland—for a period of at least 200 Myr before 200 Myr ago. It is not surprising therefore to find that some of the items appearing in *Nature* in the 1920s<sup>11</sup> indicate a favourable reception for Wegener's ideas in South Africa. It is also significant that one of these reports mentions the South African geologist A. L. du Toit. Alexander du Toit became one of the greatest advocates of continental drift, effectively taking up the banner of drift following Wegener's untimely death on the Greenland ice cap in November 1930. Ultimately du Toit published his classic text on drift—*Our Wandering Continents*—in 1937; and it was briefly reviewed for *Nature* by E. B. Bailey in 1939<sup>12</sup>.

Of the relevant items which were contributed to *Nature* during the inter-war period, two, I think, are gems: the report of a Princeton summer school, written by E. B. Bailey in 1927<sup>13</sup>, and the review of the proceedings of the Symposium on Continental Drift, held in New York in 1926, written by Arthur Holmes in 1928<sup>14</sup>. Bailey's account of the "Princeton Summer School of Geology and Natural Resources" 1927, led and organised by the legendary R. M. Field, makes fascinating reading. He is obviously fresh from, and filled with enthusiasm by, his two traverses of the North American continent. The result is a very vivid account of, and, one suspects, some very profound comments on, North American geology. At one point in describing the geology of Pennsylvania he is so struck by its similarities with the British Isles and Europe that he exclaims "It is as if the Atlantic did not exist or, in other words, as if Wegener, after all were a true prophet".

Holmes's review relates to the proceedings of the famous, if not infamous, mid-year meeting of the American Association of Petroleum Geologists, held in New York during November 1926. The meeting was subtitled "a Symposium

on the Origin of Movement of Land Masses, both Inter-Continental and Intra-Continental, as proposed by Alfred Wegener". Wegener himself was present and the North Americans in particular gave him what would be termed in their own parlance "a hard time". In fact it is often said, with relatively little exaggeration, that as a result of this meeting no North American geologist seriously considered the possibility of drift for the next 40 yr. However, this was not true of all European geologists and certainly not of southern hemisphere geologists, as noted above. Holmes himself was one of the European geologists who was not at all averse to drift but was concerned that many of the particular arguments which Wegener used did his cause more harm than good. He noted that "There is, indeed a distinct danger that the easy disproof of large sections of the Wegener hypotheses may be mistaken for a demonstration of the impossibility of continental drift as a geological process. The important issue is now not so much to prove Wegener wrong as to decide whether or not continental drift has occurred, and if so, how and when". With the advantage of hindsight this strikes one as being a very profound summary of the situation pertaining at the time, and indeed for the following forty years. Because of his own prejudice in favour of drift, Holmes had the insight to pick out some of the more constructive and enduring suggestions made at this symposium. Thus he alighted on the suggestion of van der Gracht, "that there may have been a pre-Carboniferous 'Atlantic' which was closed up by the Caledonian diastrophism" a concept which was to be formulated in much greater detail 40 yr later by Wilson<sup>17</sup> and Dewey<sup>18</sup>. Again taking his cue from van der Gracht he states his own preference for convection in the mantle as the driving mechanism for continental drift, this being apparent from his other writings at this time<sup>19</sup>. Both it seems were following the suggestion of Ampferer<sup>20</sup> and others. Holmes goes so far as to conclude that "Geophysics will not be in a position to deny continental drift until it has thoroughly explored the possibilities of convection or other currents in a highly viscous substratum, and the forces set up by the interaction of magnetic and electric fields".

Several correspondents, quite justifiably, doubted the accuracy of the geodetic measurements which Wegener claimed, substantiated that drift is occurring at the present time<sup>21</sup>. It is interesting to note that only now are relatively new instruments such as the mekometer, and new techniques, such as satellite and lunar ranging, providing the accuracy required to make direct measurements of drift. Even accurate measurement and monitoring of intra-continental crustal movements had received scant attention until the institution of the international 'Geodynamics' project in 1970 and the increased interest in earthquake prediction of recent years. Other letters bring out the limitations of fossils as indicators of continental drift and the inconclusive and often conflicting nature of the evidence provided by palaeontology<sup>22</sup>.

It is perhaps not surprising therefore to learn that very little was written about the possibility of continental drift between 1930 and 1960, that is, following the initial discussion in the wake of Wegener's book, and before the revival of the debate following the palaeomagnetic discoveries of the late 1950s. It is also noticeable, and probably significant, that the first article on continental drift to appear in *Nature* is dated 1960. Before this all the contributions were reviews, reports, letters, presidential addresses or accounts of special lectures or discussions. Clearly this could be a result of editorial policy but in fact it is true of the geological literature as a whole during this period. It is as though no self-respecting geologist in the Northern Hemisphere was prepared to risk his reputation by publishing a full-length article on continental drift.

We are not, however, left completely without written records during these years; in fact two very useful summaries of the status of the debate were given by Watts in 1935<sup>24</sup> and Good in 1950<sup>21</sup>, of which the latter is considerably less objective than the former. Perhaps one of the most pertinent statements made in this period was that of Sir Thomas Holland commenting on du Toit's demonstration of the remarkable similarities between the geology of Africa and South America from Lower Palaeozoic to Upper Mesozoic times: "If it be true that these continental masses have moved horizontally in late geological times away from one another, there is no reason why others should not have done so too, in spite of the circumstance that no completely satisfactory explanation of the mechanics involved can be offered at present. Further work on the lines so ably undertaken by du Toit is now more necessary than mathematical criticism with insufficient quantitative data regarding the physical state of the Earth's sub-crust under the continents and oceans"<sup>25</sup>.

Towards the end of the period 1930-1960 it was often said that southern hemisphere geologists found it necessary to invoke drift because of the geological record on their continents but that northern hemisphere geologists found the geological evidence for drift equivocal. Even Holmes seemed to lose some of his initial enthusiasm for drift as is apparent in the review written in 1953 (ref. 5). I always found this very puzzling in that it seemed to me that the evidence for the former continuity and juxtaposition of structures, faunal provinces and even lithologies across the North Atlantic is at least as compelling as that for the reassembly of Gondwanaland. It came as no surprise to me therefore to find that British geologists such as Bailey<sup>15</sup> and Trueman<sup>26</sup> had been saying this as much as 30 yr earlier but that their witness had largely gone unheeded. It was as though most geologists and geophysicists considered drift to be disproved because of the inadequacy of the geodetic data and mechanisms that Wegener appealed to, and the unacceptable historical association with catastrophism which was perpetuated, unfortunately, by certain writers, well into the 20s and 30s. Taylor, for example, in 1926 (ref. 27) modified his ideas by postulating the capture of the Moon in mid-Cretaceous time rather than its origin out of the Pacific but this was still too catastrophic and non-uniformitarian to satisfy the geologists<sup>16</sup>.

In spite of the fact that most geologists by the mid-1950s had rejected drift on the basis of geophysical and philosophical arguments, many geologists had from the outset acknowledged that the ultimate test of drift must come from the empirical evidence, that is, the domain of geology<sup>28</sup>. In fact, in the event, it was very empirical data which convinced most Earth scientists of the validity of continental drift, but data which derived from a marriage of geology and geophysics. Fundamental to these new discoveries was the development of the science of palaeomagnetism, the study of the intensity and direction of the Earth's magnetic field in the past as recorded in the permanent magnetism of rocks. If it is assumed that the Earth's magnetic field was essentially dipolar in the past, as it is today, then it is possible to test the hypothesis that the continents have changed their relative positions (except in the unlikely event that all relative movement has been along lines of magnetic latitude). If one makes the additional assumption that the magnetic field has been axially dipolar in the past as it is thought to have been recently, when averaged over thousands or tens of thousands of years, then clearly one can deduce the palaeolatitude of a particular continental area at any given time, and its orientation with respect to the meridian. Essentially, palaeomagnetic results confirm the hypothesis of continental drift as envisaged by Wegener and in particular vindicate the palaeolatitudes he deduced, on palaeoclimatic

grounds, for his supercontinent of Pangaea during Permo-Carboniferous times<sup>29</sup>. Detailed palaeogeographies and palaeolatitudes for particular times, however, are still somewhat equivocal because of the limitations of the method. As Sir Edward Bullard is reputed to have said, "There is nothing in this field which could not be solved by palaeomagnetism if the results were ten times more numerous and the method ten times more accurate".

The decade of the 1960s also saw the confirmation of drift in terms of a sudden and spectacular advance in our understanding of the origin and evolution of the ocean basins. The theory which set the stage for this breakthrough first appeared in print in the pages of *Nature* in 1961 (ref. 30). It was presented by R. S. Dietz who termed it "sea-floor spreading", but the idea was originally conceived by H. H. Hess, as Dietz subsequently acknowledged<sup>31</sup>. Hess's original article was circulated as a preprint in 1960 and finally appeared in print in 1962 (ref. 32). Subsequently two important riders were added to the spreading sea floor hypothesis by Vine and Matthews in 1963 (ref. 33) and by Wilson in 1965 (ref. 34). The first proffered an explanation of the pronounced and linear oceanic magnetic anomalies and the second an explanation of the abrupt offset of mid-ocean ridge crests along transverse fracture zones. Both were later substantiated; the Vine and Matthews hypothesis as a result of the definition of a geomagnetic reversal time-scale and the acquisition of more magnetic anomaly data, and the Wilson "transform fault" hypothesis as a result of improved epicentral locations and focal mechanism solutions for earthquakes occurring on the oceanic fracture zones.

It is probably true to say that for most uncommitted geologists and geophysicists the most compelling and decisive evidence in favour of continental drift was provided by the data confirming the Vine Matthews hypothesis which were presented by Pitman and Heirtzler<sup>32</sup> and Vine<sup>36</sup> in 1966. The first public presentation of these results occurred at a conference in New York in November 1966, 40 yr to the month after the fateful meeting in New York in 1926. At this meeting also, I. R. Sykes presented the new seismological evidence which provided striking confirmation of Wilson's "transform-fault" hypothesis<sup>37</sup>, thus providing additional confirmation of the hypotheses of spreading and drift. Soon after this both hypotheses were reformulated by McKenzie and Parker<sup>38</sup> and Morgan<sup>39</sup> in terms which became known as "plate tectonics". The concept of plate tectonics is itself an outgrowth of Wilson's transform fault hypothesis and the remarkable success of continental fitting at shelf edges which implies long term rigidity of vast areas of the crust and uppermost mantle.

In spite of the fact that the continental drift debate was not settled to most peoples' satisfaction until 1966 or 1967, most articles which appeared in *Nature* from 1960 onwards are favourably disposed towards it. This is not so representative of the geological literature as a whole during this period but probably reflects the fact that most of the initial palaeomagnetic results were obtained in British laboratories and as a result of this many British geophysicists were converted to belief in the theory. In addition, *Nature* seemed to attract many of the classic articles which paved the way for the ultimate proof and acceptance of the theory.

An article by Wilson in 1960 (ref. 40) provided an interesting curtain raiser to this final chapter of the continental drift debate. This article is doubly interesting because before this Wilson was in no sense a convert to drift and yet in this 'transitional' article one can see the beginnings of ideas which eventually amounted to sea-floor spreading and plate tectonics. He does this by allowing a certain amount of expansion of the Earth to account for the mid-ocean ridges. Other authors have favoured much larger scale



expansion on the assumption that the Earth was originally entirely covered by continental crust<sup>41</sup>. This would involve a fivefold increase in volume, either since the Earth was formed or since drift was initiated. Such an idea has also been championed by S. W. Carey and B. C. Heezen. It has roots which go back to the mid-nineteenth century as mentioned by Rupke<sup>4</sup>, but has never gained wide acceptance. With the advent and success of plate tectonics it now seems unnecessary and inappropriate to invoke large-scale expansion, although it has recently been revived yet again by H. G. Owen<sup>42,43</sup>.

Despite the compelling evidence which has corroborated sea-floor spreading and continental drift within the past decade certain fundamental problems regarding the detailed reconstruction of Gondwanaland have persisted. These are, notably, the position of Madagascar against Africa<sup>44</sup> and whether parts of south-east Asia should be included in Gondwana<sup>45,46</sup>. In time, presumably, both problems will be solved by further palaeomagnetic studies, and indeed this stage may have been reached with respect to Madagascar, recent palaeomagnetic results favouring a southward motion relative to Africa as originally suggested by du Toit<sup>47,48</sup>. The question of the driving mechanism for drift and plate tectonics is also equivocal although some stimulating ideas have been derived in recent years from seismic studies of lateral heterogeneity in the mantle<sup>49,50</sup> and consideration of 'absolute' as opposed to relative plate motions<sup>51,52</sup>.

I think it will be apparent from the foregoing that as regards the resolution of the continental drift debate, which was clearly fundamental to so many aspects of the science, geology stood still for essentially 40 yr, mainly it seems because geologists attached greater importance to the physical arguments rather than the geological evidence. It was as though the following remark of Eddington in 1922 fell on deaf ears, for it could equally well have been said in 1962.

"The time has gone by when the physicist prescribed dictatorially what theories the geologist might be permitted

to consider". A. S. Eddington in an address to the Geological Society of London, 21st November 1922 (ref. 53).

- <sup>1</sup> *Nature* 109, 202-203 (1922).
- <sup>2</sup> Wegener, A. *Discovery* 3, 114 (1922).
- <sup>3</sup> Robb, A. A. *Nature* 126, 841 (1930).
- <sup>4</sup> Rupke, N. A. *Nature* 227, 349-350 (1970).
- <sup>5</sup> Holmes, A. *Nature* 171, 669-671 (1953).
- <sup>6</sup> Wilson, J. T. *Proc. Amer. Phil. Soc.* 112, 309-320 (1968).
- <sup>7</sup> Cole, G. A. J. *Nature* 110, 798-801 (1922).
- <sup>8</sup> Gregory, J. W. *Nature* 115, 255-257 (1925).
- <sup>9</sup> Evans, J. W. *Nature* 111, 393-394 (1923).
- <sup>10</sup> Jeffreys, H. *Nature* 195, 448 (1962).
- <sup>11</sup> Bullard, E. C., Everett, J. E. & Smith, A. G. *Phil. Trans. R. Soc. London* A258, 41-51 (1965).
- <sup>12</sup> Carey, S. W. in *Continental Drift, A Symposium* (ed. by Carey, S. W.) (University of Tasmania, Hobart, 1958).
- <sup>13</sup> *Nature*, 109, 787 (1922); *ibid.* 110, 332 (1922).
- <sup>14</sup> Bailey, E. B. *Nature* 143, 139 (1939).
- <sup>15</sup> Bailey, E. B. *Nature* 120, 673-675 (1927).
- <sup>16</sup> Holmes, A. *Nature* 122, 431-433 (1928).
- <sup>17</sup> Wilson, J. T. *Nature* 211, 676-681 (1966).
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## articles

### Shape and pattern specification during microtubule bundle assembly

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*The ways in which control of microtubule packing, linkage, and nucleation of tubule assembly, may be related to the cross-sectional shaping of developing microtubule bundles are explored.*

In certain types of microtubule bundles the tubules are packed and linked together in precisely defined configurations. Many of these bundles are called axonemes (axle-threads) because they run along the central longitudinal axes of slender cell processes such as cilia, flagella, sperm tails, suctorian tentacles, and the axopodia of certain

rhizopods<sup>1</sup>. On the other hand, highly ordered microtubular axostyles and nemadesmata are situated in the cell bodies of certain flagellates and ciliates<sup>2</sup>. Ciliary and flagellar axonemes with the well-known nine-plus-two microtubule configuration occur in a wide range of eukaryotic organisms and cell types. All the other types of highly ordered microtubule bundles so far described have only been found in certain unicellular 'organisms' such as protozoans, phytoflagellates, and the spermatozoa of a few arthropods<sup>1,3,4</sup>.

These microtubule bundles are particularly suitable cell components for analysing control of growth and form at

both the molecular and ultrastructural levels. The tubules are often packed in simple repeating patterns and the shapes and dimensions of microtubule bundles are sometimes specified with considerable exactness. Microtubule bundles are two or three orders of magnitude larger than viruses but the highly ordered architecture of most tubule bundles seems to result, like that of certain virus coats<sup>9</sup>, from the self-assembly of large numbers of protein sub-units although only a small number of protein species are involved. Some of the control processes operating during the morphogenesis of microtubule bundles are likely to be similar to those concerned with the deployment of less compact and well ordered microtubule arrays. Such arrays are an important feature of the organisation of most eukaryotic cells and are associated with various fundamental cell activities, including anaphase chromosome movement, hormone secretion, axonal and certain other forms of intracellular transport, formation of plant cell walls, and cell shaping and locomotion<sup>10</sup>.

Does assembly proceed along basically similar lines for all microtubule bundles? The following account considers control of assembly for several types of bundles and provides some indication of the range of assembly procedures which are likely to operate. It also indicates that construction of different types of bundles involves different combinations of assembly procedures. For example, pre-fabrication of non-microtubular structures which act as templates seems to be required for direction of early stages in the assembly of some, but not all, types of microtubule bundles.

### Nucleation of tubule assembly

Two closely related<sup>11</sup> (molecular weight about 55,000) protein sub-units,  $\alpha$  and  $\beta$  tubulin, self-assemble in certain conditions to form microtubules<sup>12,13</sup>. The tubules of a microtubule bundle almost invariably start to assemble in very close proximity to each other in a particular cell region. Sometimes the number of tubules which start to assemble is specified exactly<sup>14</sup>. These observations imply that initiation of the assembly of each tubule of a bundle is nucleated *in vivo* by a special non-diffusible 'nucleating element' which is located in a particular cytoplasmic region. If this were not the case, the substantial pools of tubulin which are present in some cells before tubule assembly starts<sup>15-18</sup> should almost always polymerise to form variable numbers of randomly oriented tubules, and rarely form tubule bundles with well defined numbers of closely adjacent tubules oriented parallel to each other. The molecular

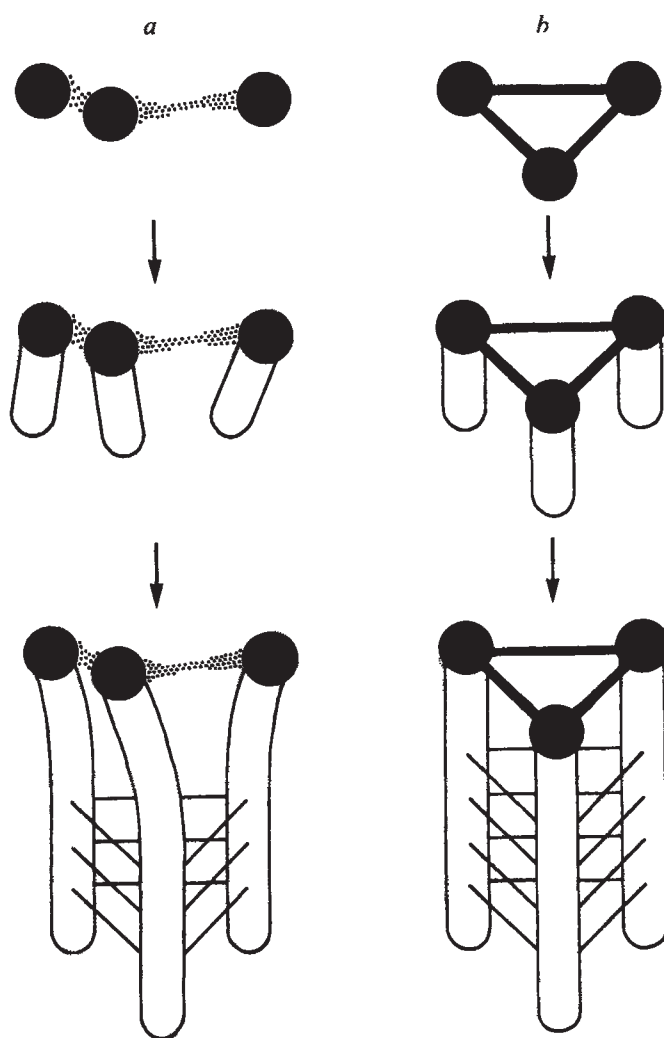
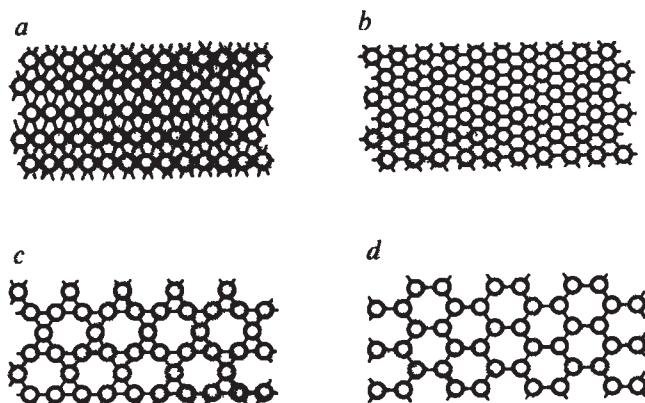


Fig. 2 Establishment of microtubule packing. *a*, The nucleating elements (black spheres) are not precisely arranged. Microtubules self-link into a regularly spaced arrangement. *b*, The nucleating elements are bound together to form an MTNT which defines the arrangement of the tubules before they are connected together by links.

Fig. 1 Equivalent patterns of tubule packing and linkage as revealed in cross-sections of tubule bundles. *a*, *Nemadesma* in the flagellate *Entosiphon*<sup>20</sup>; *b*, *nemadesma* in the ciliate *Nassula*<sup>28</sup>; *c*, axoneme in the radiolarian *Sticholonche*<sup>31</sup>; *d*, axoneme in the heliozoan *Raphidiophrys*<sup>24</sup>.



nature of nucleating elements is unknown. They differ in at least some detail from the ring-shaped tubulin aggregates which nucleate tubule assembly *in vitro*<sup>20-22</sup> because these aggregates can assemble from freely diffusible components. It is possible that the aggregates nucleate assembly of bundle tubules in *in vivo* conditions if they associate with certain non-diffusible components. If this is the case, it is the latter which represent the effective nucleating elements *in vivo*.

Cytoplasmic localisation of bundle tubule assembly is achieved because the nucleating elements are usually bound together and may be attached to the surface of some other cell component<sup>23-25</sup>. Concentrated groupings of nucleating elements and materials which connect them together have been termed microtubule-organising-centres (MTOCs) (ref. 26), nucleating sites, nucleating centres, and initiating sites<sup>27</sup>. We have adopted the term MTOC.

### Establishment of pattern

Patterns of microtubule packing in tubule bundles seem to be established in two main ways<sup>9,24,25</sup>. For some bundles, pattern seems to be defined by self-linkage<sup>9,25</sup> when inter-



tubule links connect adjacent tubules together. Links are usually peg-shaped structures with lengths of up to 40 nm and diameters of 3–7 nm. They are probably made up of protein subunits. The nexin links of cilia are largely composed of protein subunits (molecular weight 150,000)<sup>29</sup>, but no other details of the molecular composition of any other types of links are available. In several types of microtubule bundles most of the tubules are linked together in an equivalent fashion so that a repeating lattice of tubules and links is apparent when cross sections of the bundles are examined (Fig. 1). Such patterns may be established by self-linkage (Fig. 2a). This could be the case provided that the lengths of the links, and the positions and orientations at which they bind to tubule walls are precisely specified. At least some of these specifications are made in certain instances<sup>28,32,33</sup>. Analysis of axoneme assembly in certain heliozoans reveals that the pattern of tubule packing is established after nucleation of tubule assembly in a way which indicates that self-linkage is responsible<sup>24,34</sup>.

A distinctly different procedure seems to establish pattern during assembly of certain other bundles. Pattern seems to be determined by a pre-existing arrangement of nucleating elements before tubule assembly begins because tubules are precisely positioned when they first start to assemble<sup>14,28,35</sup>. An MTOC in which nucleating elements are bound together in a highly ordered arrangement before they initiate tubule

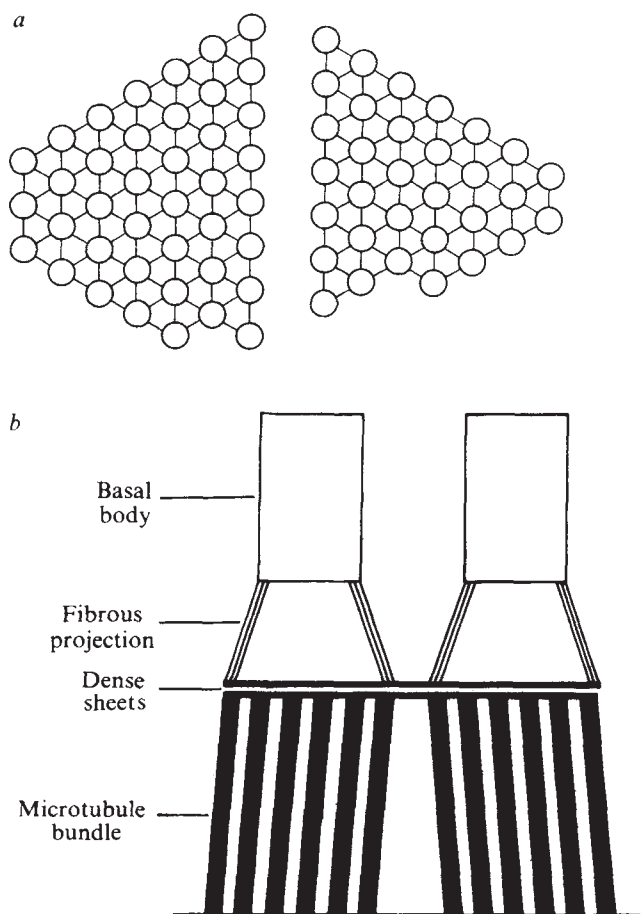


Fig. 3 The paired microtubule bundles of the ciliate *Urotricha*. *a*, Tubule arrangement and cross-sectional shape of a bundle pair based on micrographs of *U. faurei*<sup>36</sup>. *b*, Lateral view of the upper portions of a pair of bundles in *U. ovata*<sup>37</sup> showing attachment to basal bodies.

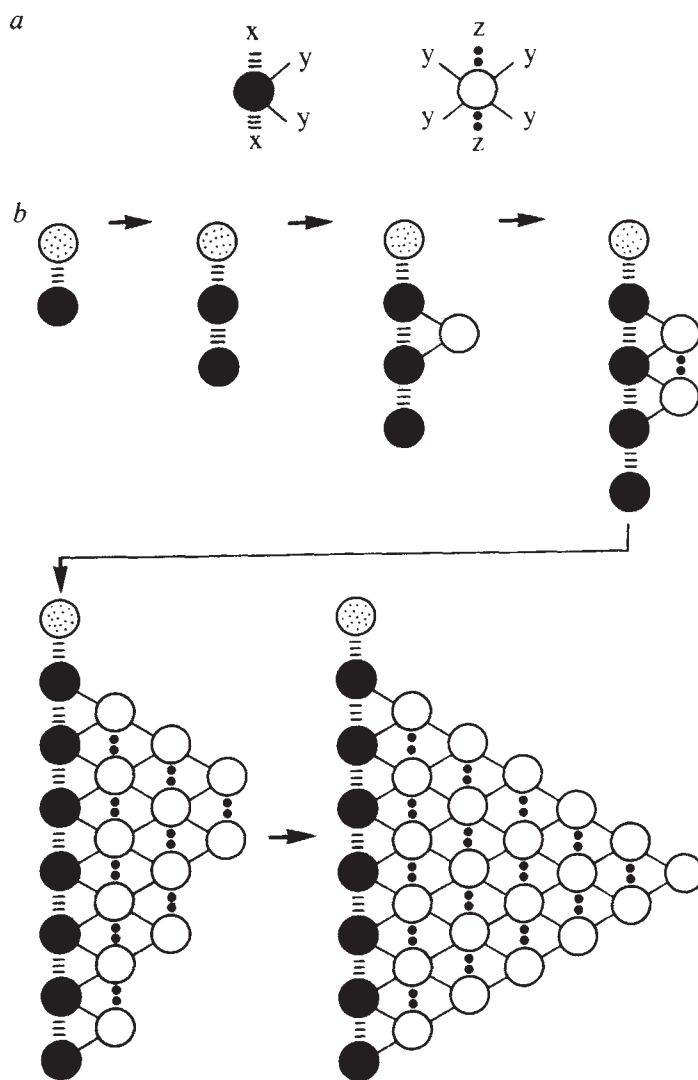


Fig. 4 A model scheme for assembly of a triangular MTNT. *a*, Schematic representation of the two types of nucleating elements required showing the arrangement of their binding points which lie in the same plane and can only bind to points of the same type on other elements. *b*, Stages in the assembly of elements to form the MTNT on a basal body projection. Each basal body which is attached to the top of a bundle has a projection (stippled) which bears an x binding point. An x bond can form between a basal body projection and any black element which is not already bound to any other element, and can also join two black elements provided one of the elements is already bound to another black element or a basal body projection. The y bonds can only join unbound or 'free' white elements to two other elements (black or white) which are already bound together so that two y bonds can form simultaneously when binding is effected. A z bond forms when both of the white elements it is to connect are already joined to the template by y bonds.

assembly is termed a microtubule-nucleating-template (MTNT) (ref. 28). There is no reason why such a template should not self-assemble from proteins coded for by chromosomal genes in the absence of any pre-existing copies of the template; these templates need not be regarded as genetically autonomous self-replicating entities. Although the pattern of microtubule packing is apparently maintained by self-linkage as tubules grow out from an MTNT (Fig. 2b), there may be advantages in assembling an MTNT to establish certain types of microtubule patterns and these are considered in the sections which follow.

## Triangular shape specification

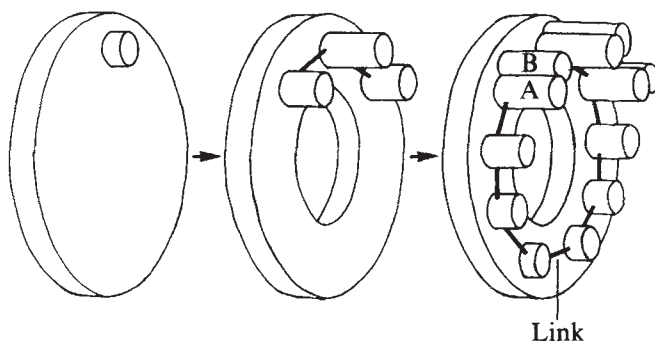
The cytopharyngeal microtubule bundles (nemadesmata) of certain ciliates have rather precisely defined cross-sectional shapes although their tubules are apparently linked together in an equivalent hexagonal fashion. For example, in *Urotricha* the bundles have cross-sectional profiles which closely approximate to equilateral triangles in shape. Furthermore, bundles are closely juxtaposed in pairs and their cross-sectional profiles are precisely oriented with respect to each other (Fig. 3a). The tops of each of the bundles in a pair are attached to two sheets of dense material which are connected by fibrous projections to the proximal ends of a pair of basal bodies (Fig. 3b). No information is available about the sequence of events which occurs during bundle morphogenesis in *Urotricha* but in related ciliates the tubules of similar bundles grow out from dense sheets attached to basal bodies<sup>25,38</sup>.

Specification of the cross-sectional shape and orientation of a bundle is most readily accounted for in theory by assuming that an MTNT forms by the assembly of two types of nucleating elements. If the conditions outlined in Fig. 4 are satisfied assembly of a triangular template will proceed along the lines indicated. The steric conformation of the elements and their binding points must be such that binding always results in all y bonds projecting from the same side along the row of black elements, and such that the points on the surfaces of the elements where nucleation of tubule assembly starts are all facing in the same direction (perpendicular to the lower surface of the template which faces away from the basal body). If the number of black elements available for assembly is limited, this number will largely determine the size of the template even when an excess of white elements is present. In these conditions the assembly scheme is a closed one like that for the coats of icosahedral viruses<sup>39</sup>. The orientation of the cross-sectional profiles of adjacent bundles with respect to each other may be determined by pre-existing spatial information in the form of precise orientation of the two basal bodies and their projections. There is considerable evidence that adjacent basal bodies in the ciliate cortex are often oriented with this degree of precision<sup>40</sup>.

An important feature of the use of an MTNT in the situation described above is that all the bundle tubules can be identical. Each tubule would be potentially capable of linking to six neighbours hexagonally arranged around it. The extent to which this potentiality is realised depends on the number of neighbours specified by the MTNT. The possibility that triangular shaping could be established by self-linkage of two types of tubules along the lines suggested above for nucleating elements cannot, however, be excluded. Nevertheless, it is difficult to see how two types of tubules bound to sheets of dense material could have sufficient freedom of movement to self-link and form adjacent bundles with triangular cross sections oriented with respect to each other, unless tubule positions are specified fairly exactly by an MTNT at the start of tubule assembly.

## Hollow axonemes with circular cross sections

The circular arrangement of microtubules in the axonemes of cilia and flagella is established at the beginning of basal body morphogenesis when exactly nine tubule triplets start to assemble in a regular circular arrangement. In *Paramecium* the sequence shown in Fig. 5 seems to occur. The dense disk may be an MTNT with nucleating elements bound in an evenly spaced circular arrangement around its perimeter. It is possible, however, that links can act as nucleating elements in this and certain other situations. When, and if this ever occurs, unlike a self-linkage pro-

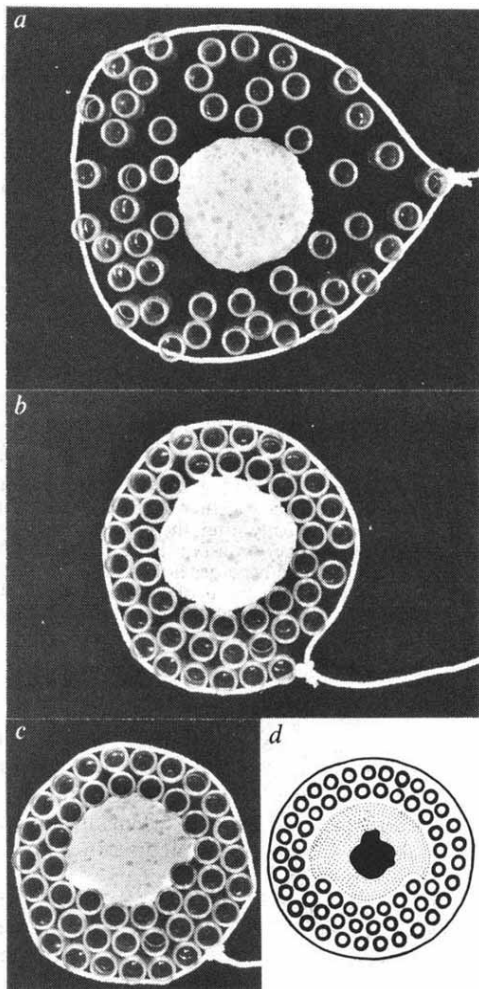


**Fig. 5** Schematic diagram showing early stages in basal body assembly in *Paramecium* (ref. 41). One of the A tubules of a basal body starts to assemble shortly before the other eight. A second and third A tubule then assemble on either side of the first one and this sequence continues until a closed circular array has formed. Links connect tubules together at an early stage of their assembly and disappear shortly after the circular array is complete. The tubules grow out from a flat dense structure which is initially disk-shaped and then becomes hollowed out at its centre. Addition of the B tubule components of each triplet to A tubules starts before all the A tubules have formed.

cedure, tubule position is determined when tubule assembly is nucleated. For example, there may be only one nucleating element on the disk. Each of the links may be able to bind at one end at a particular orientation to certain points on existing A tubules and when it has done so nucleate assembly of a new tubule at its other end. Such a scheme accounts for the sequence of basal body tubule assembly which also occurs in several other organisms besides *Paramecium* (ref. 42). In some of these other organisms, however, a cartwheel structure with a hub and nine evenly spaced radial spokes is apparent before any of the tubules can be detected. These cartwheels may act as MTNTs if the tip of each spoke nucleates assembly of a tubule triplet<sup>43,44</sup>. Tubules and/or nucleating elements also seem to become grouped around a central non-microtubular structure during initial stages in the assembly of some other types of hollow axonemes which are circular in cross section. For example, when assembly of tentacle axonemes in the suctorian *Choanophrya* begins, tubules take up positions around a cylindrical 'condensation centre'<sup>45</sup>. This centre breaks down later in axoneme development when the circular tubule array is complete and tubules are joined together by links.

The arrangement adopted by microtubules in sperm tails of the coccid insect *Planococcus* provides an example of a situation in which a rather precise microtubule configuration may be generated simply by crowding tubules closely together around a cylindrical structure without the assembly of an MTNT or very precise control of tubule linkage. During outgrowth of sperm tail axonemes in this and certain other coccids, microtubules are closely packed (sometimes into an approximately hexagonal array) around a cylindrical core of chromatin and other dense material<sup>46,47</sup>. In *Planococcus* two concentric tube-shaped arrays of microtubules are arranged around the dense core. A curved sheet-like array is usually situated inside the completely tubular ones and the dense core is often slightly indented where this array lies against it. One way in which this arrangement could be established, in this case by a 'close packing effect', is shown in Fig. 6. Examination of other microtubule bundles also indicates that patterns of microtubule packing which are hexagonal or nearly hexagonal, and certain types of dislocations in such arrays, may be established and/or maintained by a close packing effect<sup>28,48</sup>. The origin and





**Fig. 6** Microtubule positioning in sperm of the coccid insect *Planococcus citri*. *a*, Dynamic model showing how tubules may be closely packed around a central cylinder of spongy material. Unstoppered glass specimen tubes stand on a smooth surface and are positioned fairly evenly around a cylinder of artificial sponge (the same height as the tubes). *b*, A string lariat is tightened and the tubes pack closely around the sponge. *c*, As tightening continues two circular arrays of tubes are formed and an inner curved sheet-like array presses into the sponge to produce an indentation like that in the dense core of the sperm. *d*, Diagrammatic cross section of a sperm based on electron micrographs<sup>17</sup>. Microtubules are arranged around a core of chromatin (black) and other dense material (stippled).

nature of the forces which might be involved in packing the tubules closely together in such situations are unknown.

## Non-equivalent packing patterns

More complex events than any of those considered above may be involved during the assembly of microtubule bundles in which tubules are not all linked together in an identical fashion<sup>8,34</sup>. For example, in axonemes of centroaxoplastid radiolarians approximately two thirds of the tubules are linked to two neighbours, the others are linked to three<sup>31</sup>. In some such instances, the number of tubules in a bundle is exactly defined<sup>39</sup>. Descriptions of the assembly sequences for most bundles with 'non-equivalent' patterns of tubule linkage are not yet available. It is not clear whether all the tubules in these bundles are identical so that MTNTs are required to establish pattern, or whether pattern is

generated when tubules of two or more types self-link. Greater freedom of movement will be required for tubules of two or more types to self-link than when only one type of tubule is involved. Can tubules of two or more types be confined in a localised region of cytoplasm and still have sufficient freedom of movement to effect self-linkage? A non-equivalent pattern of tubule packing seems to be established by self-linkage during generation of the double-spiral pattern in axonemes of the heliozoan *Echinospaerium* (ref. 34). In this organism, however, tubules may be free to diffuse through the cytoplasm more extensively than is usually the case because the number, size, and spacing of axonemes does not seem to be very precisely defined. In addition, the suggestion<sup>30</sup> that relatively long-range allosteric interactions take place between adjacent tubules through their links during self-linkage raises the possibility that tubules which are initially identical can self-link in a non-equivalent fashion.

More information about the molecular composition of nucleating elements, intertubule links and MTOCs will facilitate further understanding of spatial control during assembly of microtubule bundles. Progress in this field is also impeded by our ignorance of the ways in which links bind to tubules. Whether they bind directly to tubulins or to other proteins<sup>51-53</sup> which may be bound to tubule walls is unknown. There are indications that in certain flagellar microtubules some of these proteins are mainly associated with particular protofilaments (rows of tubulins) along one side of each tubule<sup>53</sup>. Such unilateral protein arrangements may help to specify patterns of link attachment.

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# Chemical modification of DNA polymerase phosphoprotein from avian myeloblastosis virus

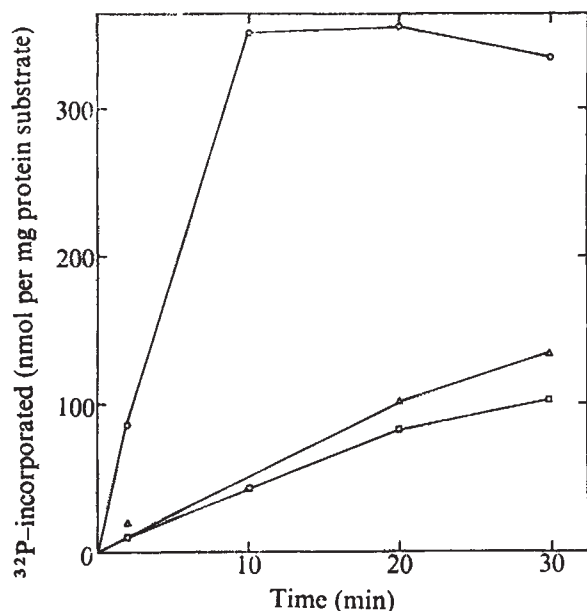
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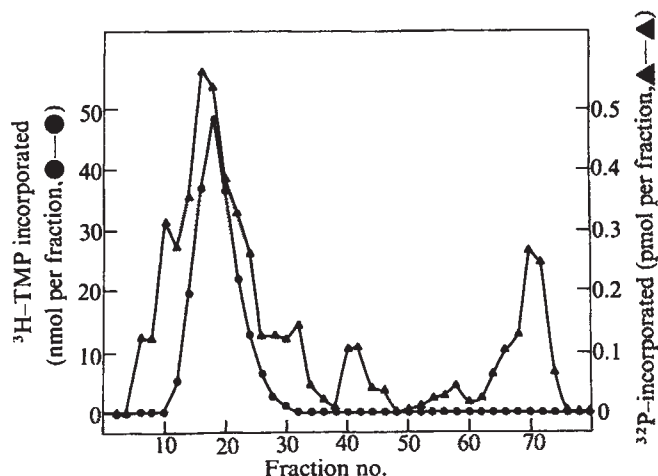
*Fractionation of purified avian myeloblastosis virus DNA polymerase, after phosphorylation in vitro, revealed the presence of a small acidic protein, a phosphate acceptor polypeptide with high specific activity. Its presence in the phosphorylated form with the polymerase resulted in as much as a 10-fold increase in the rate of DNA synthesis. Its presence in the dephosphorylated form with the polymerase had no effect in the rate of DNA synthesis.*

SINCE the recognition of an RNA-dependent DNA polymerase (RDDP) in RNA viruses<sup>1-3</sup>, much effort has been expended on studies of its structure and function<sup>1-11</sup>. Two polypeptides,  $\alpha$  and  $\beta$ , with molecular weights of 60,000 and 100,000, respectively<sup>5-8</sup>, are generally demonstrable in preparations of RDDP from avian myeloblastosis virus (AMV). But the existence of the separate components and/or interrelationships between them remain obscure. The work reported here has revealed a constituent which appears in preparations of the highly purified

RDDP activity isolated from AMV. This material has the properties of a nondialysable, small, acidic phosphoprotein, designated  $\phi$ , which can be phosphorylated by a highly purified AMV protein kinase *in vitro*. Demonstration of  $\phi$  is based on the separation of phosphorylated  $\phi$  from purified AMV-RDDP by three different methods: (1) molecular sieving on Sephadex G-200; (2) isoelectric focusing on polyacrylamide gels, and (3) the use of sodium dodecyl sulphate-polyacrylamide gels. In addition, differences in the influence of phosphorylated and dephosphorylated  $\phi$  on the rate of DNA synthesis by the polymerase are documented here. Finally, implications with respect to possible chemical modification of AMV-RDDP as a regula-



**Fig. 1** AMV protein kinase-catalysed phosphorylation in the presence of  $\gamma$ -<sup>32</sup>P-ATP and protein substrates of viral and non-viral origin. A 20-fold standard reaction mixture of AMV protein kinase was prepared containing 25 mM Tris-Cl (pH 8.3), 20 mM MgCl<sub>2</sub>, 10 mM dithioerythritol (DTE), 20 mM KCl, 0.02% (v/v) Triton X-100, 0.2 mM  $\gamma$ -<sup>32</sup>P-ATP (475 c.p.m. per pmol), and 20  $\mu$ l of AMV protein kinase<sup>12</sup>. The amounts of phosphate acceptor protein per reaction were as follows: ○, 7  $\mu$ g of purified AMV-RDDP; △, 200  $\mu$ g of partially purified AMV-RDDP; □, 2,500  $\mu$ g of  $\alpha$ -casein (Sigma). The reaction was carried out at 35 °C. At the times indicated, 10- $\mu$ l samples were placed on GF/C disks and treated as described before<sup>21</sup>. Radioactivity was determined in a Packard Tricarb liquid scintillation spectrometer and converted to nmol of <sup>32</sup>P incorporated per mg of protein substrate.



**Fig. 2** Chromatographic analysis of AMV polymerase after phosphorylation with AMV kinase<sup>12</sup>. At the completion of the protein kinase reactions described in Fig. 1, all reaction products were dialysed against buffer A (200 mM potassium phosphate (pH 7.2), 0.1 mM EDTA, 2 mM DTT, 0.2% Triton X-100 containing 10% glycerol) for 36 h with four changes of buffer to remove  $\gamma$ -<sup>32</sup>P-ATP. 0.5 ml (a fourth of the protein kinase reaction with purified AMV-RDDP as the phosphate acceptor) was subjected to chromatography by molecular sieving on Sephadex G-200 (1.6 × 60 cm) equilibrated with buffer A. Fractions of 1.8 ml were collected and analysed for <sup>32</sup>P-radioactivity (▲) as described in Fig. 1. RDDP activity (●) was determined after incubation for 60 min at 35 °C of a 10- $\mu$ l sample with 50  $\mu$ l of RDDP standard reaction mixture (50 mM Tris-HCl (pH 8.3), 0.5 mM <sup>3</sup>H-TTP (16.6 c.p.m. per pmol), 6 mM MgCl<sub>2</sub>, 0.2 mM poly(A)<sub>n</sub>·dT<sub>12-18</sub>(10:1), 40 mM KCl, containing 100  $\mu$ g of bovine serum albumin per ml), with 50- $\mu$ l samples from each reaction placed on GF/C disks and treated as described before<sup>21</sup>.

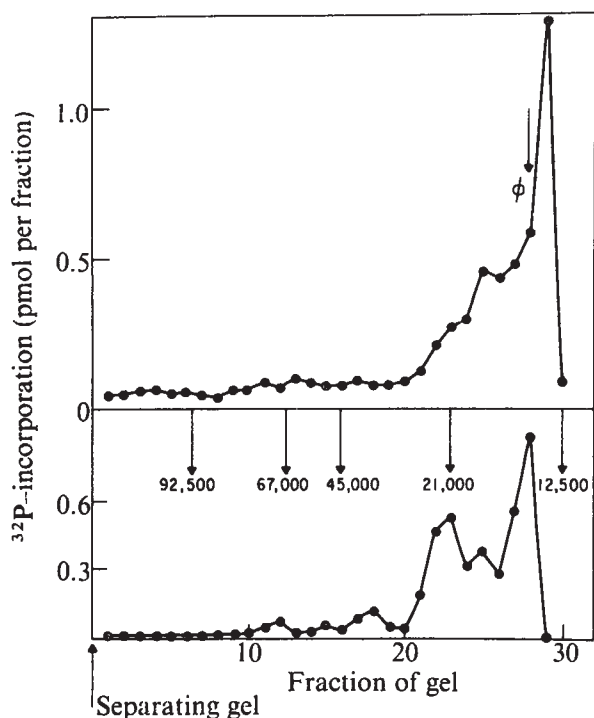
tory mechanism in the life cycle of the virion and its behaviour in the process of replication observed *in vitro* are discussed.

## Phosphorylation of polypeptides and separation of $\phi$

The kinetics of the phosphorylation reactions presented in Fig. 1 are expressed as incorporation of radioactivity from  $\gamma$ -<sup>32</sup>P-ATP per mg of protein used as the phosphate acceptor in that reaction. All phosphorylation reactions described here

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**Fig. 3** Sodium dodecyl sulphate-polyacrylamide gel electrophoretic profiles of  $^{32}\text{P}$ -radioactivity and standard proteins. Electrophoresis in the presence of 0.1% sodium dodecyl sulphate was carried out on columns ( $6 \times 120$  mm) of 15% polyacrylamide separating gel, including 6% polyacrylamide stacking gel as described by Laemmli<sup>23</sup>. Protein samples were precipitated at 4 °C by addition of trichloroacetic (TCA) and (final concentration 10%), collected by centrifugation (20,000g, 20 min), washed with acetone and suspended in sample buffer. When  $\beta$  mercaptoethanol was added, samples were heated for 2 min to 100 °C to dissociate metastable aggregates and a 0.3-ml sample was applied to each polymerised gel. Electrophoresis was carried out at 60 V until the sample passed the stacking gel, and was then increased to 120 V for about 5 h at 18 °C. In gels intended for staining, the proteins were then precipitated during 1 h at 60 °C with 14% TCA, and the gels were then washed at room temperature with 10%, 5% and 3% TCA within 24 h. These gels were then stained in a 0.05% solution of Coomassie brilliant blue R-250, in a mixture of methanol-acetic acid-water (45:9:46) during 2 h, followed by washing in the same solvent until the background was colourless. The gels were then placed in 9% acetic acid to reswell, after which they were photographed and/or traced. Gels intended for measurement  $^{32}\text{P}$ -radioactivity distribution, were sliced (collecting three slices per fraction) and solubilised, and radioactivity distribution determined as described before<sup>24</sup>. The molecular weight of AMV-polypeptides was calculated from their migration relative to proteins of known molecular weight, as described<sup>25</sup>. Upper graph, migration of  $^{32}\text{P}$ -radioactivity distribution from an aliquot of IEF pool 1 (which focused with an average  $pI$  of about 4). Lower graph, standard proteins; phosphorylase  $\alpha$  (92,500 daltons), bovine serum albumin (67,000 daltons), ovalbumin (45,000 daltons),  $^{32}\text{P}$ - $\alpha$  casein (21,000 daltons) and cytochrome  $c$  (12,500 daltons). In addition, the distribution of  $^{32}\text{P}$ -radioactivity from an aliquot of IEF pool 2 (which focused with an average  $pI$  of 7.0–7.5 in association with RDDP).

were carried out with our most purified fraction of AMV protein kinase<sup>12</sup>.

On the basis of values of nmol of  $^{32}\text{P}$ -incorporation per mg of protein substrate present in the non-viral and viral polypeptides examined, one of the virion polypeptides  $\phi$  found in association with our most purified RDDP fraction displayed the greatest specific activity of phosphorylation (Fig. 1 and ref. 12).

Fractionation, by molecular sieving on Sephadex G-200 (Fig. 2), of an aliquot from this protein kinase reaction, in the presence of our most purified RDDP (Fig. 1), followed by measurement of the distribution of RDDP activity and  $^{32}\text{P}$ -radioactivity, revealed that about 82% (Fig. 2 fractions

10–25) of the total  $^{32}\text{P}$ -radioactivity on the column coincided with the RDDP activity. Most of the remaining  $^{32}\text{P}$ -radioactivity eluted just behind myoglobin (fractions 65–75). Fractionation by isoelectric focusing on polyacrylamide gels<sup>12</sup> of another aliquot used for gel filtration as above, followed by measurements of the distribution of RDDP activity and  $^{32}\text{P}$ -radioactivity, revealed that most of the  $^{32}\text{P}$ -radioactivity on the gel focused with an average  $pI$  of about 4.0. The remaining  $^{32}\text{P}$ -radioactivity on the gel focused with an average  $pI$  of 7.0–7.5 in association with RDDP (ref. 12).

Isoelectric focusing of the enzyme resulted in considerable losses of RDDP activity. Incomplete dissociation of  $\phi$  protein from RDDP proteins is due to intramolecular salt link(s) between the phosphorylated (anionic) site(s) of  $\phi$  and neighbouring basic (cationic) amino acid(s) of RDDP  $\alpha$  and/or  $\beta$  polypeptides, since in high salt complete dissociation resulted<sup>12</sup>. Furthermore, in denaturing conditions (Fig. 3), complete dissociation of  $\phi$  protein from  $\alpha$  and  $\beta$  polypeptides was demonstrated (see later).

Electrophoresis of a sample identical to those used for gel filtration and isoelectric focusing revealed (Fig. 3) that all the  $^{32}\text{P}$ -radioactivity migrated faster than  $\alpha$  casein and slower than cytochrome  $c$  (Fig. 3, upper graph). No detectable radioactivity was observed in the regions of the gel where  $\alpha$  and  $\beta$  polypeptides from purified RDDP would be expected with molecular weights of about 60,000 and 100,000 respectively. On the basis of the distance of migration of several marker proteins (Fig. 3, lower graph), the phosphoprotein ( $\phi$ ) found in association with purified RDDP was estimated to have a molecular weight of about 15,000. Although these results demonstrate association of  $\phi$  with purified RDDP and complete dissociation of  $\phi$  from RDDP, they do not exclude the possibility that  $\phi$  is produced by limited proteolysis of the RDDP real enzyme (holoenzyme) at some time in the life cycle of virions and/or during purification<sup>13–16</sup>.

### Effect of *in vitro* phosphorylated and dephosphorylated $\phi$

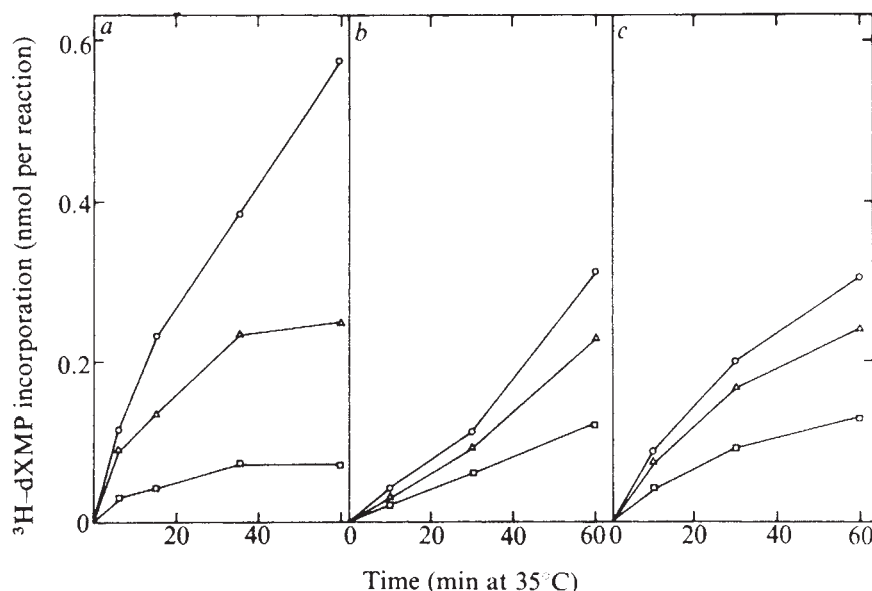
Purified AMV-RDDP was incubated for 30 min at 4 °C in the absence and presence of a preparation of purified phosphorylated  $^{32}\text{P}$ - $\phi$ . Aliquots of each sample were then used to measure rates of  $^3\text{H}$ -dGMP incorporation directed by poly(C)<sub>n</sub>-dG<sub>12–18</sub> at the times indicated (Fig. 4a). In the presence of phosphorylated  $\phi$ , the rate of poly(C)<sub>n</sub>-dG<sub>12–18</sub>-directed,  $^3\text{H}$ -dGMP incorporation increased as much as 5–10-fold (Fig. 4a). Addition of larger amounts of phosphorylated  $\phi$ , however, inhibited the rate of  $^3\text{H}$ -dGMP incorporation (results not shown). When phosphorylated  $\phi$  was added to purified AMV-RDDP reactions directed by poly(A)<sub>n</sub>-dT<sub>12–18</sub> (Fig. 4b) or AMV RNA-dT<sub>12–18</sub> (Fig. 4c), the rates of polymerisation increased but not as much.

Dephosphorylated  $\phi$  was made as described in Fig. 5. Most purified AMV-RDDP was incubated for 30 min at 4 °C in the absence and presence of dephosphorylated  $\phi$  together with the controls designated in Fig. 5. Removal of  $P_i$  from  $\phi$  eliminated the observed stimulatory effect of phosphorylated  $\phi$  (Fig. 4). This is taken to mean that AMV phosphoprotein phosphatase has essentially completely dephosphorylated  $\phi$ . In other experiments on the dephosphorylation of  $\phi$  with alkaline phosphatase or AMV phosphoprotein phosphatase, incomplete dephosphorylation was observed (results not shown). The influence of phosphorylated and dephosphorylated  $\phi$  on the rate of acid solubilisation of  $^3\text{H}$ -poly(A)<sub>n</sub> by purified AMV-RDDP from the hybrid homopolymer  $^3\text{H}$ -poly(A)<sub>n</sub>-poly(dT)<sub>n</sub> has been examined and the results will be reported elsewhere.

### Implications

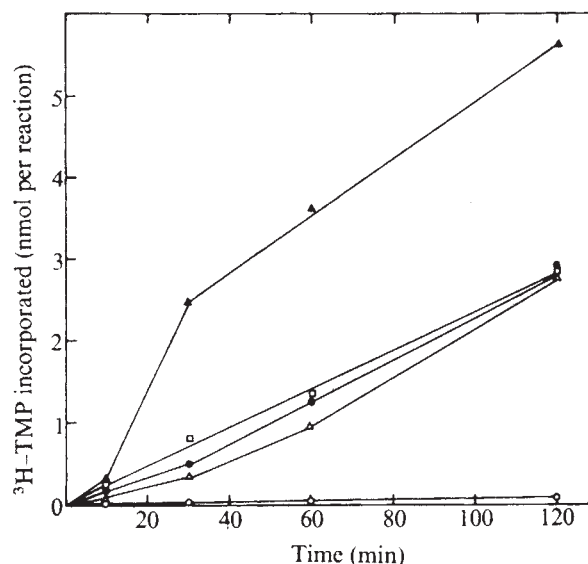
My results are relevant to several questions about the nature of RDDP. (1) What polypeptide(s) constitute the real enzyme? Reported results<sup>7–9,15</sup> support the notion that one of the two polypeptides ( $\alpha$ ) with a molecular weight of about 70,000 from

**Fig. 4** Incorporation of radioactivity by a purified fraction of AMV-RDDP in the absence and presence of  $\phi$ . 66 ng of the enzyme was incubated in the absence and presence of 2  $\mu$ l and 5  $\mu$ l of  $\phi$  at 4 °C for 30 min, and all samples were made up to 12  $\mu$ l with buffer A. After 30 min of pre-incubation of the above samples, 5- $\mu$ l aliquots were added each to a 100  $\mu$ l of the reaction mixtures described below and incubated at 35 °C for the time designated. *a*,  $^3\text{H}$ -dGMP incorporation directed by 0.2 mM poly(C)<sub>n</sub>. dG<sub>12-18</sub>. *b*,  $^3\text{H}$ -TMP incorporation directed by 0.2 mM poly(A)<sub>n</sub>. dT<sub>12-18</sub>. The homopolymers above were at a molar ratio of 10 adenosine to 1 thymine, and 10 cytosine to 1 deoxyguanosine, respectively. *c*,  $^3\text{H}$ -TMP incorporation directed by 14.8 A<sub>260</sub> AMV RNA per reaction in the presence of 0.2 mM dT<sub>12-18</sub>. At the times indicated, 10- $\mu$ l aliquots were placed on GF/C disks, treated, and the rates of  $^3\text{H}$ -XMP incorporation were determined as described in Fig. 2.  $\square$ , AMV-RDDP activity in the absence of  $\phi$ ;  $\Delta$ , in presence of 2  $\mu$ l  $\phi$ ;  $\circ$ , in presence of 5  $\mu$ l of  $\phi$ .

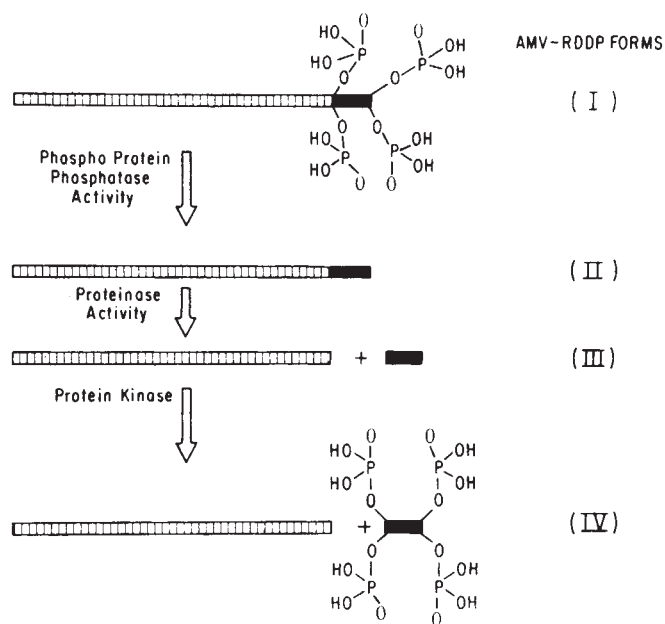


AMV is a proteolytic degradation product<sup>8,15,36</sup>, from ( $\beta$ ) polypeptide which has a molecular weight of about 110,000. This possibility is supported by demonstrations that animal viruses contain proteinase activity involved in the cleavage of precursors of polypeptides (similar to those) found in the mature virions<sup>13-16</sup>. Specific proteolytic degradation has also been demonstrated with several DNA and RNA enzymes in prokaryotes<sup>17-20</sup>. (2) Why is the DNA product small<sup>1-10</sup>? Published reports<sup>1-11</sup>, show that something was missing from the enzyme

preparations used in previous studies. In 1972 Leis and Hurwitz described the isolation and characterisation of a heat labile, nondialysable factor from AMV that stimulated DNA synthesis, but no further studies have been reported. (3) Why does AMV-RDDP exhibit low fidelity when copying a variety of polynucleotide templates<sup>11</sup>? The high error rate of AMV-RDDP has been attributed to the nature of the polymerase<sup>11</sup>. Mizutani and Temin<sup>34</sup>, however, have emphasised the importance of the reaction conditions in determining error-prone DNA polymerases in cell-free studies, and have suggested that changes in the physiological environment or altered DNA polymerases



**Fig. 5** Incorporation of radioactivity by a purified fraction of AMV-RDDP, in the absence and presence of phosphorylated and dephosphorylated  $\phi$ . Dephosphorylated  $\phi$  was made as described below. 3.5  $\mu$ g of the enzyme was incubated for 30 min at 4 °C as indicated below, and all samples were made up to 60  $\mu$ l with buffer A. After 30 min of pre-incubation, all samples were placed in ice and 10  $\mu$ l of each was added to 100  $\mu$ l of the standard RDDP reaction mixture as described in Fig. 2, and incubated at 35 °C for the time designated. At the times indicated, 20- $\mu$ l aliquots were placed on GF/C disks, treated, and the rates of  $^3\text{H}$ -TMP incorporation were determined as described in Fig. 2.  $\Delta$ , AMV-RDDP alone;  $\blacktriangle$ , AMV-RDDP in presence of  $^{32}\text{P}$ - $\phi$ ;  $\square$ , AMV-RDDP in presence of dephosphorylated  $\phi$ ;  $\circ$ , dephosphorylated  $\phi$  alone;  $\bullet$ , AMV-RDDP in presence of bacterial alkaline phosphatase. Phosphorylated  $\phi$  that had been dialysed to remove orthophosphate against Tris buffer was dephosphorylated with 20  $\mu$ g of alkaline phosphatase in Tris-HCl (50 mM, pH 8.0) and  $\text{MgCl}_2$  (5 mM). Dephosphorylation was carried out for 30 min at 35 °C. A sample equivalent to 5  $\mu$ l of the phosphorylated  $\phi$  and 5  $\mu$ l of the dephosphorylated  $\phi$  were used in each case.



**Fig. 6** Hypothetical model on the sequential changes in AMV-RDDP during its purification and/or at some time in the life cycle of the virion.

are responsible for the high mutation rates observed<sup>11</sup>. The results reported here support the suggestion that altered DNA polymerases in the life cycle of the virion and/or during purification are responsible for the high infidelity observed<sup>11,34</sup>. This possibility can be tested by examining the influence of  $\phi$  on the fidelity of AMV-RDDP enzyme in a cell-free system.



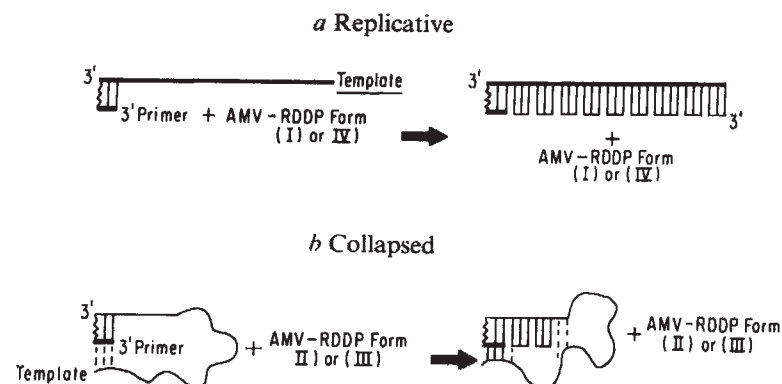


Fig. 7 A model to account for the relatively small size (5–10S) and unfaithful nature of the products observed with AMV-RDDP and other oncornaviruses. *a*, Forms I and IV of AMV-RDDP (see Fig. 6) contain  $\phi$  a large oligophosphoprotein, which helps to maintain the template structure in the replicative form during DNA synthesis. *b*, Forms II and III of AMV-RDDP (see Fig. 6) contain the dephosphorylated form of  $\phi$ , which permits the replicative nature of the template to convert into the collapsed form, by means of intramolecular hydrogen bonding<sup>26</sup>.

## Model of possible enzymatic modification of RDDP

The model shown in Fig. 6 predicts that AMV-RDDP may exist in four forms at some time in the life cycle of the virion and/or during purification of the polymerase. This is facilitated by the combination of three enzyme activities—phosphoprotein phosphatase, proteinase and protein kinase. Association of these activities with purified virions from avian myeloblastosis has been established (refs 12, 13, 14, 16, 27 and unpublished results). Form IV of AMV-RDDP is strongly supported by the results reported here.  $\phi$  is the phosphate acceptor protein with the highest specific activity in a purified AMV-RDDP preparation (Figs 1–3). Whether  $\phi$  and AMV-RDDP are ever covalently linked (AMV-RDDP form I) remains to be seen. The existence of form I of AMV-RDDP is so attractive that it requires further experimentation. Demonstration of the existence of form I will assist in the documentation of the possible existence of forms II and III. The results presented in Fig. 5 support the existence of form III. In addition, I have observed a difference in the stability between AMV-RDDP activity in lysates buffered with phosphate (no loss of activity in 48 h at 4 °C) and lysates buffered with Tris-HCl (76% loss). Furthermore, when such lysates were subjected to isoelectric focusing, specific polypeptides from lysates buffered with phosphate had f1s more acidic than those observed in lysates buffered with Tris-HCl (ref. 37 and unpublished results).

I conclude that this is due to the inhibition of AMV phosphoprotein phosphatase<sup>12</sup> by phosphate, a potent inhibitor of phosphatases. Depending on the procedures used to purify AMV-RDDP and whether or not throughout purification and storage of the enzyme, the solutions are buffered with Tris-HCl or phosphate buffer, the amount of phosphorylated  $\phi$  may vary considerably and thus influence the yields, properties and form(s) of the enzyme isolated. There is support for this in several published reports<sup>30–33, 37</sup>.

In this respect it is interesting that in enzyme preparations buffered with Tris-HCl throughout purification only 10–20% recovery was observed<sup>30, 31</sup>, whereas in enzyme preparations buffered throughout purification with phosphate, as much as 70–90% recovery has been reported<sup>32–33, 37</sup>. It is also interesting that studies *in vitro* on the infidelity of AMV-RDDP were conducted with enzyme preparations maintained throughout purification and stored in solutions buffered with Tris-HCl (ref. 11). Furthermore, dephosphorylation of purified AMV-RDDP with alkaline phosphatase from *E. coli* resulted in a decrease in the rate of polymerisation<sup>37</sup>. Similar results have been reported with RSV-RDDP (ref. 28).

I propose a model of AMV-RNA replication which may account for the small size (5–10S) of the products<sup>4, 10</sup> and infidelity of replication of RDDP (ref. 11). This is represented diagrammatically in Fig. 7b. RDDP will replicate only when the polymerase (forms II or III, Fig. 6) is present because  $\phi$  is

dephosphorylated in both these forms, so that the template exists in the collapsed form by means of intramolecular hydrogen bonding<sup>26</sup>. In contrast, Fig. 7a shows the replicative form of RNA in the presence of AMV-RDDP forms I or IV. This is made possible by the presence of the phosphorylated  $\phi$ , which helps the template to remain so, while the polymerase makes the complete and faithful cDNA. One of the predictions in this model (Fig. 7a) has far-reaching implications concerning the size and fidelity of the polymerase product. In appropriate conditions, AMV-RDDP form IV, which can now be made available, may be able to make complete and faithful copies of the RNA from these virions.

The activity of RDDP from murine and simian C-type RNA viruses in the presence of phosphorylated  $\phi$  increased by as much as 10-fold. No increase in the DNA polymerase activity from *E. coli* and *Mycrococcus luteus* could be detected in the presence of phosphorylated  $\phi$  (C.M.T. in preparation). A comparable specificity of bacterial protein factors towards their homologous polymerase has been reported<sup>38</sup>. The pronounced enhancement observed on the rates of polymerisation catalysed by the polymerases from avian, murine and simian RNA viruses in the presence of phosphorylated  $\phi$ , implicates  $\phi$  as an important constituent in the replication of the genetic information of RNA viruses.

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# Structure of an antibody combining site by magnetic resonance

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*The structure of the combining site of the DNP binding IgA mouse myeloma protein MOPC 315 has been determined by a combination of high resolution nuclear magnetic resonance, electron spin resonance, model building and chemical modifications. This approach yields the general dimensions of the site, its polarity and asymmetry features, the assignment of the DNP-contact residues and their three-dimensional coordinates.*

ONE of the central problems in immunochemistry is the structural basis of antibody specificity; how does such a high diversity in molecular recognition reside in what seems to be a family of proteins closely related in their chemical structure? Recent progress in X-ray analysis of Fab fragments of homogeneous myeloma proteins has led to the elucidation of the three-dimensional structure of the binding site of two antibodies: the human protein New which binds vitamin K and the mouse protein McPC 603 which binds phosphorlycholine<sup>1,2</sup>. From these studies it is clear that the residues forming the sites that are complementary to the antigen are contributed by the hypervariable regions<sup>3</sup> (three segments of 5–10 residues each, in both light and heavy chain) and that replacements of amino acid residues in these segments will generate binding sites with new specificities. Such replacement will not disturb the 'immunoglobulin fold' of the variable domains which remain similar in all antibodies. Thus on a background of a common three-dimensional structure a vast number of specificities can be generated. This diversity in recognition is the essence of the immune system and therefore a comparative analysis of a large number of combining sites is required in order to provide the details of molecular recognition by antibodies. It is also important to develop new methodologies by which the structure of the antibody site can be analysed in solution and yield information comparable with that obtained from crystal studies. We describe here an attempt to elucidate the structure of the binding site of the DNP binding protein 315, a mouse IgA myeloma protein<sup>4</sup>, by a combination of high resolution nuclear magnetic resonance (n.m.r.), electron spin resonance (e.s.r.), model building and chemical modification. We show that by this approach it is possible to determine the general dimensions of the site, its polarity and asymmetry features and to assign the contact residues with the ligand as well as their three-dimensional coordinates.

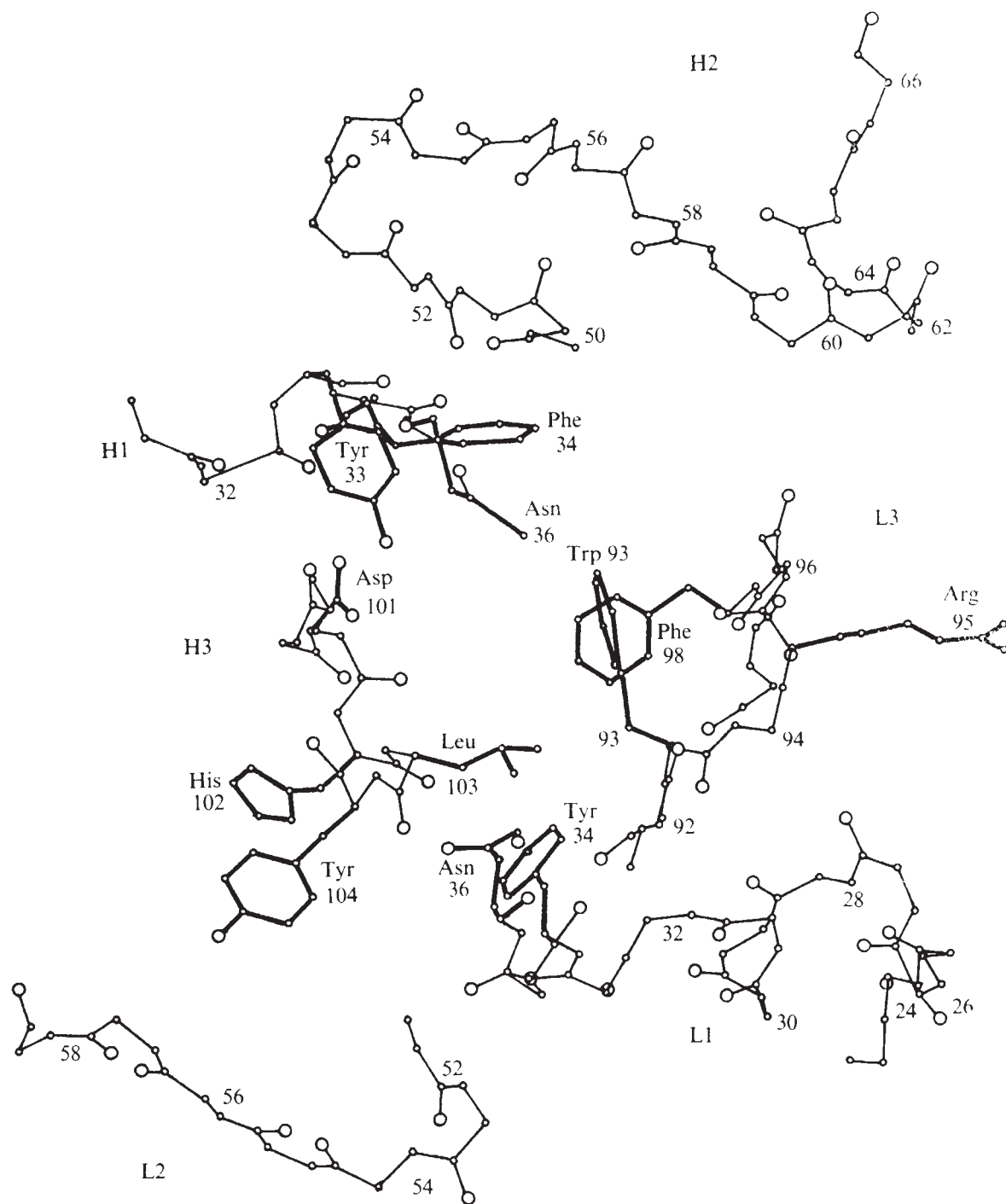
## Model building as a basis for structural analysis

A quantitative comparison of the tertiary structure of human and mouse variable domains has demonstrated that the immunoglobulin fold is very similar in different variable domains while structural differences occur predominantly in the hypervariable loops which comprise the combining site<sup>5</sup>. Hence the variable region can be regarded as consisting of a rigid framework to which are attached the hypervariable loops. This provides a basis for comparative analysis of antibody combining sites by model building, which then uses the coordinates of the framework from X-ray analysis data of known immunoglobulins and the sequence of the immunoglobulin which is to be analysed. Considering the diversity of immunoglobulins and the impracticality of X-ray analysis of all interesting antibodies this model building approach is an attractive way for a comparative study of antibody combining sites, provided the resulting combining site and the binding of ligand to it can be tested by various physical methods. Some of the features of the combining site of protein 315 have already been indicated by Poljak on the basis of comparison with the structural model of protein New (ref. 7). More recently, Padlan *et al.*<sup>8</sup>, have described the model building of the site of protein 315 on the basis of coordinates derived mainly from protein 603 and using also information from protein New. The resulting combining site<sup>8</sup> is presented in Fig. 1 and is the subject of further refinement on the basis of the n.m.r. data presented in this article. The main feature of 315 combining site is its hydrophobic nature. The clustering of hydrophobic residues is more extensive than in either protein 603 or New. The middle of the surface produced by the hypervariable loops is dominated by a pronounced cavity which can accommodate the dinitrophenyl ligand and the depth of this cavity is approximately 1.2 nm. Details of this combining site are discussed by Padlan *et al.*<sup>8</sup>. At first sight it seems that there are several different modes of binding that can accommodate the ligand in it. We shall demonstrate both that the n.m.r. results dictate a unique arrangement of the DNP ligand in the cavity of the protein 315 and can be used to refine the three-dimensional positions of various residues in the site. We have also probed that part of the combining site which binds the side chain of DNP ligand by using homologous series of spin-labelled haptens or alkyl phosphonate derivatives of haptens.

## Gross architecture of the combining site

Since approximately 50% of the binding energy of DNP ligands to protein 315 is contributed by the hapten side chain<sup>9</sup>, it is of great interest to study the environment of





**Fig. 1** The predicted combining site of MOPC 315. This has been obtained from the coordinates of Padlan *et al.*, which have been very kindly made available to us before publication. The hypervariable loops of the heavy (H) and light (L) chains and the hapten binding site are indicated.

the combining site in this area. To do this we have used two series of haptens of systematically increasing chain length with magnetic resonance probes attached. One series consists of either six- or five-membered nitroxide rings attached to the DNP moiety which is analysed by the e.s.r. spectra and the other series consists of alkyl phosphonate groups attached to the DNP moiety which are analysed by their phosphorus n.m.r. signals.

The spin label mapping procedure involves comparison of the observed e.s.r. hyperfine splittings with those expected as a result of different postulated motions for the spin label. An indication of the type of analyses has been given previously for DNP ligands containing a six-membered

nitroxide ring<sup>10</sup>. From that study, it emerged that the DNP ring is rigidly held in the combining site, that the depth of the site is 1.1–1.2 nm and that the minimum lateral dimensions at the entrance to the site are 0.6 × 0.9 nm. However, this method has now been extended to the analysis of five-membered spin-labelled haptens with chiral centres. Measurements of such haptens result in separate bound signals from each enantiomer<sup>11</sup> and this shows that the site is asymmetric with respect to the plane of the DNP ring. The two enantiomers which probe different sides of the site are restricted differently in their motions. Comparison of the experimentally measured values of the maximum hyperfine splittings,  $A_{\max}$  with those calculated on the basis

**Table 1** Deduction of lateral dimensions of the combining site by comparison of experimental values of the esr hyperfine splitting constants with those expected for possible motions of the five-membered spin label haptens as deduced from studies of molecular models.

| Hapten                                                                                                         | Allowed motion†<br>(about the bond shown) | Spatial requirements for<br>this motion (nm)† |        | Calculated maximum<br>value of hyperfine<br>splitting constant<br>for allowed motion | Observed maximum hyperfine<br>splitting constant |              |
|----------------------------------------------------------------------------------------------------------------|-------------------------------------------|-----------------------------------------------|--------|--------------------------------------------------------------------------------------|--------------------------------------------------|--------------|
|                                                                                                                |                                           | width                                         | height |                                                                                      | Enantiomer 1                                     | Enantiomer 2 |
| DNP-NH-CH <sub>2</sub> -  N-O | ±40                                       | 0.7                                           | 1.0    | 2.81                                                                                 | 3.2                                              | 2.7          |
| 5-F-DNP-NH-  N-O              | ±20                                       | 0.6                                           | 0.9    | 3.1                                                                                  | 3.0                                              | 2.63         |

\* Chiral centre.

† Calculated from atomic models.

of intrinsic allowed motions of these haptens (Table 1) assuming a rigid DNP ring leads to the determination of precise lateral dimensions. These conclusions are summarised in Fig. 2.

### Environment of hapten side chains

The polarity of the nitroxide radical's environment can affect the magnitude of the hyperfine splitting. The relatively large difference in  $A_{\max}$  between the 'rigid' enantiomers of haptens V (3.2) and VI (3.0) (~0.2 mT, Table 1) is expected to arise from a polarity effect, that is the nitroxide of hapten V is probably close to a positive charge which results in a higher value of  $A_{\max}$ . A positive charge 0.1–0.3 nm away from the NO and along its axis could account for the difference in the values of  $A_{\max}$  (refs 12, 13) between the two haptens. The length and geometry of hapten V would then allow the positively charged residue to be placed as shown in Fig. 2. Reference to the predicted model (Fig. 1) immediately suggests that this residue must be Arg 95<sub>L</sub>, since hapten V is rigid and the NO lies along the axis of the DNP ring.

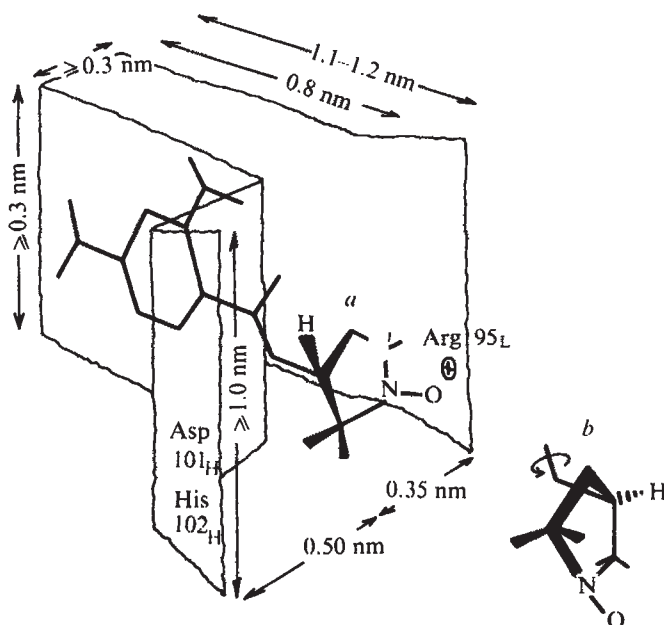
The use of <sup>31</sup>P magnetic resonance provides an additional and more powerful probe of electrostatic environment. Since the  $pK_a$  of a phosphonate grouping is extremely sensitive to neighbouring charges, changes in the ionisation state of <sup>31</sup>P groupings with pH can be relatively easily monitored by observation of the changes in <sup>31</sup>P chemical shifts. The phosphonate grouping approximates to a hemisphere of radius 0.3 nm so that by using a series of side chains of different lengths it is possible to probe different areas of the combining site for positively and negatively charged groups.

The  $pK_a$  values of three TNP alkyl phosphonate haptens (methyl, ethyl and propyl) when either free in solution or when bound to the Fv have been measured from titrations of <sup>31</sup>P chemical shifts (at 129 MHz). Only the TNP propyl phosphonate has its  $pK_a$  significantly affected, being decreased by 0.8 unit (from 7.7 to 6.9) suggesting the proximity of positively charged group. Using molecular models the extended length of this hapten measured along the plane of the TNP ring is 1.2 nm. From the dimensions of the site obtained from the e.s.r. mapping studies this places the phosphonate grouping just beyond the entrance to the combining site and in a position to interact with the positively charged group detected by the e.s.r. studies. The  $pK_a$  mapping and the e.s.r. mapping techniques are therefore complementary and reference to the model confirms that Arg 95<sub>L</sub> is the candidate for this positive charge in both cases. Lys 52<sub>H</sub> is another positively charged residue at the edge of the combining site as has been confirmed by affinity labelling with bromoacetyl DNP

lysine<sup>14</sup>. However, consideration of the geometries of the Tnp phosphonate haptens and the affinity label indicate that the phosphonate ligands are considerably shorter and will not be able to interact with Lys 52<sub>H</sub>. This further illustrates how different techniques can be used to probe different areas of the combining site.

The predicted model places one (His 102<sub>H</sub>) out of the three histidines in the Fv fragment near the combining site. His residues are particularly convenient to study by high resolution n.m.r. The  $pK_a$  values of the three histidine residues in the Fv fragment have found to be 5.9, 6.9 and 8.2 (ref. 10). On hapten binding, only one His residue has its  $pK_a$  significantly affected, the  $pK_a$  being increased from 6.9 to 7.2–7.4 depending on the hapten side chains. The narrow linewidths of the His resonances of this histidine suggest that it is on the surface of the protein and readily accessible to solvent. Reference to the model suggests this can only be His 102<sub>H</sub>. This means that the  $pK_a$  value of 5.9 can be assigned to His 97<sub>L</sub>, since we have previously assigned a  $pK_a$  value of 8.2 to His 44<sub>L</sub>, from the results of paramagnetic spin label difference spectroscopy<sup>10</sup>. His 102<sub>H</sub>

**Fig. 2** The overall dimensions of the DNP binding site as determined by spin-label mapping. The two enantiomers of one of the five-membered spin-labelled haptens are shown. Anomer *a* is rigidly immobilised but anomer *b* is free to undergo a restricted rotation as shown.





therefore provides a convenient marker of the edge of the site. His 102<sub>H</sub> on the other hand cannot be the candidate for the positive charge discussed previously which is sensed by the spin labels and the phosphonate groups. This is because the  $pK_a$  of His 102<sub>H</sub> is similarly affected by various DNP ligands regardless of their length or charge. Hence the change in His 102<sub>H</sub>  $pK_a$  reflects local changes in its environment whereas Arg 95<sub>L</sub> interacts specifically with charged groups of the hapten which have the appropriate length of the side chain.

### The DNP binding site

The red shift in the optical absorbances of DNP ligands bound to protein 315 (ref. 5) and circular dichroism studies<sup>15</sup> indicate interaction between the DNP ring and a tryptophan residue. The complex formed between a DNP ligand and L-tryptophan was therefore used as a model for the DNP-Fv interaction in an n.m.r. analysis. The addition

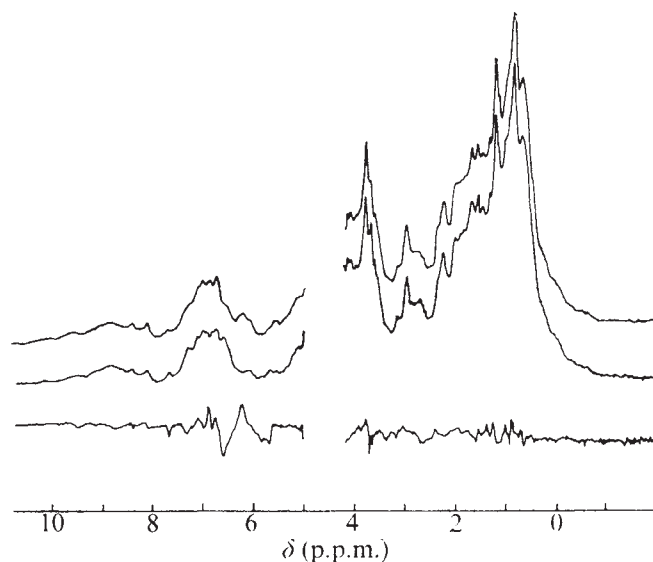


Fig. 4 The 270 MHz proton spectra of the Fv fragment alone and in the presence of Dnp aspartate and the difference spectrum between the two (enlarged  $\times 4$ ). Conditions were (Fv) = (Dnp-aspartate) = 1.15 mM, pH = 6.9,  $T = 303$  K (0.15 M NaCl).

of varying amounts of DNP-aspartate into a 10 mM solution of L-tryptophan results in upfield chemical shifts of all the aromatic resonance. By appropriate titrations<sup>16</sup> estimates of the fully bound shifts of all the protons in the complex can be obtained. These are in the range 0.1–0.5 p.p.m. upfield. Replacing tryptophan by any of the other aromatic amino acids does not result in any observed shifts thus showing the specificity of the interaction between the DNP ligand and tryptophan. The upfield chemical shifts can be analysed in terms of ring current interactions<sup>17–19</sup> which relate the shift on a nearby nucleus to its position from the centre of its aromatic ring responsible for the shift. Since all the aromatic protons in this system are shifted upfield this immediately suggests that a stacking interaction is occurring. The structure of the DNP and TNP complex is then found by a computer search which tests all possible

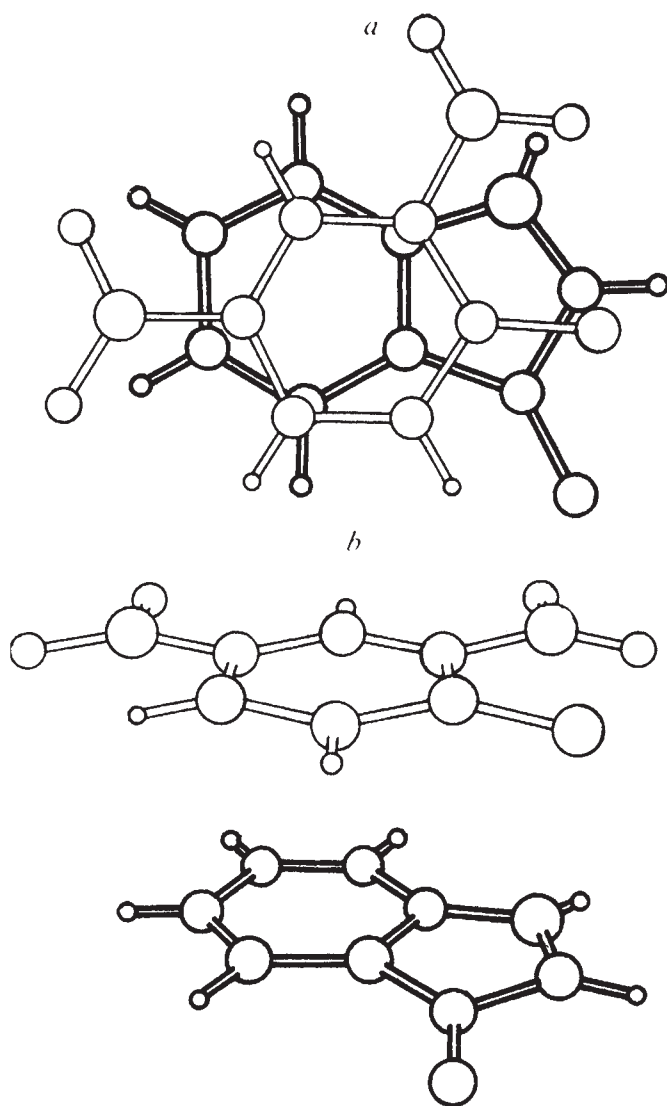


Fig. 3 The structure of the DNP-aspartate and tryptophan complex as deduced from ring current interactions. The two views are obtained from a computer search of the possible structures, with a separation between the two rings of 0.33 nm, which give calculated ring current shift ratios in best agreement with the experimental values. The shifts are calculated using the Johnson-Bovey equation modified to take into account the indole ring. The two views represent (a) a vertical view down the  $z$  axis and (b) the vertical view rotated 30° about the  $z$  axis and 60° about the  $x$  axis.

Table 2 Chemical shifts ( $\delta_0$ ) and the change in chemical shift ( $\Delta\delta$ ) of resonances perturbed on binding of DNP-aspartate to the Fv from MOPC 315 at pH 6.9

| Hapten resonances                   | $\delta_0$ (p.p.m.) | $\Delta\delta$ (p.p.m.) | No. of protons |
|-------------------------------------|---------------------|-------------------------|----------------|
| H <sub>a</sub>                      | 9.18                | -1.68                   | 1              |
| H <sub>b</sub>                      | 8.36                | -2.3                    | 1              |
| H <sub>c</sub>                      | 7.04                | -1.31                   | 1              |
| -CH <sub>2</sub> -                  | 2.76                | -0.1                    |                |
| (Complex pattern with 3 main peaks) | 2.91                | -0.2                    | 2              |
| -CH-                                | 2.99                | -0.26                   |                |
|                                     |                     | not observable          |                |
| Protein resonances                  |                     |                         |                |
| 5.9 His C2                          | 7.82                | -0.06                   | 1              |
| —                                   | 7                   | broadens out            | ~2             |
| 5.9 His C4                          | 6.95                | -0.07                   | 1              |
| —                                   | 6.9                 | -0.19                   | 1              |
| —                                   | 6.62                | 0.08                    | 1              |
| —                                   | 6.37                | 0.295                   | 2              |
| —                                   | 6.33                | -0.175                  | <2             |
| —                                   | 6.16                | -0.255                  | 2              |
| Peak decreases as hapten is added   | 7.2                 |                         | ~2             |
| Peak increases as hapten is added   | 7.42                | poss. 0.22              | 2              |

\* Positive sign is downfield, negative sign is upfield shift change. Measurements are made at 270 MHz, at  $T = 303$  K and in the presence of 0.15 M NaCl. Shifts were measured from the sodium salt of 3-(trimethyl-silyl-propane) sulphonic acid, as an external standard.

The addition of varying amounts of DNP-aspartate to the Fv results in changes in the chemical shifts of the proton resonances at 270 MHz from both hapten and protein. The initial and final spectra are shown in Fig. 4, together with the difference spectrum between Fv and the 1:1 complex. If the histidine resonances are used as internal area markers only about 10 aromatic protons are perturbed on hapten binding. By means of difference spectroscopy the titration behaviour of all these resonances can be followed and the results are given in Table 2. On the protein, apart from the resonances assigned to a histidine (97,  $pK_a=5.9$ ) five protons have their resonances shifted up-field. The magnitude of these shifts are very similar to the DNP-induced ring current shifts of the tryptophan protons in the model complex. It seems reasonable to conclude that some, if not all, of the resonances in the protein which

are shifted upfield on addition of the hapten are from a tryptophan and that the DNP ring when bound in the Fv will interact with this tryptophan in a very similar manner to that shown in Fig. 3. Inspection of the proposed binding site in Fig. 1, immediately suggests that this tryptophan in the Fv is Trp 93<sub>L</sub>.

The upfield shifts of the DNP protons when bound to the Fv are far larger than those in the DNP-aspartate-tryptophan complex. Analysis of these shifts reveals that it is necessary to invoke a minimum of two additional aromatic residues to explain the magnitude of these shifts. One of these aromatic residues must be above and in almost van de Waal's contact with the H(5) and H(6) protons while a second aromatic residue is required to account for the shift on the H(3) proton. Reference to model suggests that these two residues are Phe 34<sub>H</sub> and Tyr 35<sub>L</sub>. Hence the DNP ring is essentially enclosed in an 'aromatic box' consisting of Trp 93<sub>L</sub>, Phe 34<sub>H</sub>, and Tyr 34<sub>L</sub>.

The binding of DNP-glycine to the Fv provides the clue that now allows the orientation of the hapten in the site to be fixed. The side chain-CH<sub>2</sub>-protons of this hapten experience a shift of 1 p.p.m. on binding to the Fv. To explain this shift it is necessary to introduce a fourth ring in the vicinity of the DNP situated about 0.3 nm above the CH<sub>2</sub> grouping of DNP-Gly. This can only be Tyr 33<sub>H</sub> which occupies similar position in the model. It is interesting that nitration of this tyrosine does not affect the binding constant of haptens to protein 315 suggesting that the interaction between Tyr 33<sub>H</sub> and the hapten is through the ring and not the hydroxyl. In contrast, in protein 603 an H bond

so as to point out of the site in which event Tyr 104<sub>H</sub> will point into it and can then interact with Tyr 34<sub>L</sub>. This is also compatible with the lack of any large change in methyl resonance in Fv on hapten binding.

Other differences from the predicted model also arise from consideration of a 'second layer' of aromatic residues. For instance the protons assigned to Trp 93<sub>L</sub> are themselves ring current shifted upfield from the normal expected positions in a protein. This suggests that Trp 93<sub>L</sub> points back into the site where the protons of the six-membered ring can be upfield shifted by Phe 98<sub>L</sub>. Finally the positioning of the hapten allows conclusions to be drawn about possible H-bonding schemes. The two nitro groups on the hapten seem to be necessary for strong binding and suggest some H-bonding interactions to amino acid side chains<sup>9</sup>. The only possible H-bonding groups which can interact with NO<sub>2</sub> groups on the DNP ring positioned as discussed above are Asn 36<sub>L</sub> and Tyr 34<sub>H</sub>. Interestingly both these H bonds as well as the involvement of Trp 93<sub>L</sub> are on the light chain and may be the reason for a weak binding site on light chain<sup>22</sup> and the V<sub>L</sub> dimer<sup>23</sup> for DNP ligands. The refined model of the combining site modified according to the data in this paper is shown in Fig. 5 and illustrate the considerable agreement with the original model. However, the n.m.r. results allow very precise positioning of the contact residues and given the assumptions that aromatic rings are the only source of upfield shift interactions and that there is no motional averaging of these rings, the placing of the rings around the binding site is estimated to be accurate to  $\pm 0.05$  nm.

Table 3 Comparison of combining site residues which interact with ligands\*

| Protein | Ligand            | H1                                             | H2                                     | H3                                        |
|---------|-------------------|------------------------------------------------|----------------------------------------|-------------------------------------------|
| 315     | DNP               | 31 Ser Gly <i>TYR</i> Phe Trp <sup>†</sup> Asn | 50 <i>PHE</i> Ile <i>LYS</i> Tyr       | 59 99 Asp Asn Asp His — — — — — Leu       |
| 603     | Phosphorylcholine | Asp — Phe <i>TYR</i> Met <i>GLU</i>            | Ala Ser <i>ARG</i> Asn..... <i>GLU</i> | <i>ASN</i> Tyr Tyr Gly Ser Thr <i>TRP</i> |
| New     | Vitamin K         | Asn — Asp Tyr Tyr Thr                          | <i>TYR</i> Val Phe Tyr                 | Asx LEU ILE ALA — — — — — Gly             |
|         |                   | L1                                             |                                        | L3                                        |
| 315     | DNP               | 32 Ser Asn <i>TYR</i> Ala Asn                  |                                        | 92 Leu <i>TRP</i> Phe <i>ARG</i> Asx His  |
| 603     | Phosphorylcholine | Lys Asx Phe Leu Ala                            |                                        | X X X X X                                 |
| New     | Vitamin K         | <i>GLY ASN</i> His Val Ser                     |                                        | Ser <i>TYR</i> Asp Arg <i>SER LEU</i>     |

\* Interacting residues (italic) are according to refs 1 and 2 for New and 603 respectively and from this work for protein 315. When no residues are marked the possibility of interacting with peptide chain backbone is not excluded. H1, H2, H3, L1 and L2 are hypervariable regions of H and L chains. X, unknown residue due to incomplete sequence. The numbering of sequences is according to protein 315 with homology maximised. We thank Professor R. J. Poljak for the revised sequence of protein New residues 31-35 before publication.

<sup>†</sup> Changed from lysine after partial resequencing (D. Givol, personal communication).

is formed between Tyr 34<sub>H</sub> and the phosphate group of the phosphoryl choline hapten.

Consideration of geometry of DNP glycine then shows that the H(3) proton is on the opposite side of the molecule to the CH<sub>2</sub>. Thus the H(3) proton must be over Tyr 34<sub>L</sub> while the H(5) and H(6) protons are in van de Waals' contact with Phe 34<sub>H</sub>. Further refinement of the environment of Tyr 34<sub>L</sub> comes from the observation of an Overhauser effect<sup>12</sup> on the H(3) proton of the DNP ring. Irradiating the protein resonances in the Fv at 6.6 p.p.m. results in the reduction of the signal intensity of this H(3) proton. This implies that one or more protons on Tyr 34<sub>L</sub> (which have resonances at this position) are within 0.3 nm of the H(3) proton. Further the affected protons on Tyr 34<sub>L</sub> must be influenced by another aromatic ring since the resonance at 6.6 p.p.m. is shifted upfield by  $\sim 0.6$  p.p.m. from the position expected for an unperturbed tyrosine residue. According to the predicted model this other aromatic ring can only be Tyr 104<sub>H</sub> but this necessitates repositioning this residue so as to be vicinal to Tyr 34<sub>L</sub>. This is only possible if the position of Leu 103<sub>H</sub> is altered in the postulated model

Several interesting points emerge from the comparison of the combining site of protein 315 with those of protein New and 603. In Fab' New-vitamin K<sub>1</sub>OH complex, the naphthoquinone ring of vitamin K<sub>1</sub>OH is in close contact with Tyr 90<sub>L</sub><sup>1</sup> (Tyr 93<sub>L</sub> in Table 3). Sequence homology shows that Trp 93<sub>L</sub> in protein 315 which interacts with the DNP is homologous to Tyr 90<sub>L</sub> in New, and therefore this provides an interesting example for a change in specificity on replacing one residue in the site. Note that protein 315 also binds menadione in lower affinity than DNP (ref. 24), but protein New does not bind DNP ligands. Charge interactions play a dominant part in the binding of protein 603 to the hapten phosphoryl choline<sup>2</sup>. On the other hand, another model building study on rabbit anti-type III polysaccharide (BS-5) suggest clustering of the hydroxyamino acids Tyr, Ser and Thr and also Asn in the combining site which can form H bonds with hydroxyls of the sugar ligand<sup>25</sup>. It is clearly seen that some pattern of changes in the antibody binding site can be deduced from such comparative studies as illustrated in Table 3.

The magnetic resonance study of interaction between



haptens and antibody provides a functional sense to the structure predicted by model building. It is possible to assign interactions to residues in the site and also to determine their correct orientation with respect to the hapten. The positioning of Trp 93<sub>L</sub>, Phe 94<sub>H</sub>, Tyr 34<sub>L</sub>, Asn 36<sub>L</sub>, Arg 95<sub>L</sub> and other residues allows understanding of the specificity of binding DNP ligands to protein 315 and indicates that this approach when applied to other antibodies will provide information about the structural basis of antibody specificity.

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# letters to nature

## Acceleration of charged particles to extremely high energies in pulsar far fields

SINCE the inception<sup>1</sup> of the pulsar magnetosphere, it has been realised that charged particles, streaming away from the neutron star beyond the light cylinder, would attain high energies. Goldreich and Julian<sup>1</sup> calculated the potential drop  $\Phi_M$  across the open field lines at the polar cap of the star, noting that this was the order of the maximum energy that such particles could attain.  $\Phi_M$  is of order  $10^{16}$  V for the Crab pulsar. They estimated (their equation (34)), however, that the energy actually reached would be very much less than this. I show here that in fact all escaping particles will reach energies of order  $\Phi_M$ .

We assume that the electromagnetic field is given adequately by the force-free approximation<sup>2,3</sup>, in which particle mass is neglected, and then calculate the motion of charges in this field to first order in the mass. This leads to an estimate of the region of adequacy of the approximation.

If the mass is neglected altogether, the motion is simply

$$\mathbf{v} = \mathbf{U}_E + \mathbf{v}_{||}\mathbf{B}/B \quad (1)$$

where  $\mathbf{v}_{||}$ , the component along the magnetic field  $\mathbf{B}$ , is unrestricted. In force-free theory<sup>4</sup>, the electric field,  $\mathbf{E}$ , is given by

$$\mathbf{E} = -\mathbf{v}_R \times \mathbf{B} \quad (2)$$

where  $\mathbf{v}_R$  is the co-rotation velocity  $\Omega \times \mathbf{r}$ ,  $\Omega$  being the angular velocity of the star. Thus the electric drift  $\mathbf{U}_E$  is

$$\mathbf{U}_E = \mathbf{E} \times \mathbf{B}/B^2 = \mathbf{v}_R - (\mathbf{v}_R \cdot \mathbf{B})\mathbf{B}/B^2 \quad (3)$$

At large radial distances,  $\mathbf{U}_E$  becomes radial<sup>5-7</sup>, and equal to  $c$  (within the force-free approximation). In the general, non-axisymmetric case<sup>7</sup>, the far field is dominated by an electromagnetic wave (of frequency  $\Omega$ ), for which  $E/B = c$ , so this result is clear.

It is essential within the approximation that  $\mathbf{U}_E$  should not

exceed  $c$ . The Lorentz factor  $\gamma_E$  corresponding to  $\mathbf{U}_E$  follows from (3):

$$\gamma_E^2 = B^2/\mathbf{B} \cdot \tilde{\mathbf{B}} \quad (4)$$

$$\text{where } \mathbf{B} = (1 - \mathbf{v}_R^2/c^2)\mathbf{B}_p + \mathbf{B}_t \quad (5)$$

The derived field  $\mathbf{B}$  is of fundamental importance in force-free theory<sup>2,3</sup>. We require therefore for the validity of the approximation, that

$$\mathbf{B} \cdot \tilde{\mathbf{B}} \geq 0 \quad (6)$$

It follows from the universal spiral structure of the far field<sup>7</sup> that whereas  $B^2$  decreases as  $r^{-2}$ ,  $\mathbf{B} \cdot \tilde{\mathbf{B}}$  decreases as  $r^{-4}$ . There is a great degree of arbitrariness at present in the directional dependences of the quantities, but we assume that there exists a class of solution for which (6) is valid. At large  $r$  therefore,

$$\gamma_E \approx F r/r_1 \quad (7)$$

where  $r_1$  is the light radius  $c/\Omega$ , and  $F$  is some function of direction.

To zero order in the mass therefore, the particles gain energy in proportion to their distances from the star. To find out when inertial forces will curb this process, we must calculate the first order 'guiding centre drift'. This depends partly on  $\mathbf{v}_{||}$ , calculation of which in general<sup>8</sup> necessitates integration of an equation for  $d\mathbf{v}_{||}/dt$ . Here, this is unnecessary because of the existence of an exact integral of the motion due to Edean<sup>9</sup>,

$$\mathcal{E} = \gamma(1 - \mathbf{v}_R \cdot \mathbf{v}/c^2) \quad (8)$$

This arises from the requirement that the whole system be stationary in the co-rotating reference frame. When  $\mathbf{v}$  is inserted from (1) into (8), there results a quadratic equation for  $\mathbf{v}_{||}$  whose coefficients depend on  $\mathbf{B}$  and  $\mathcal{E}$ . For values of  $r$  sufficiently large that (7) is adequate, the physical root of this equation, and

the associated value of  $\gamma$  are approximately followed by equation (9):

$$v_{||}/c \approx -(\sigma \mathcal{E}/(\sin \theta F))(r_1/r)^2, \quad (9)$$

$$\gamma \approx \gamma_E,$$

where  $\theta$  is the polar angle, and  $\sigma$  is the sign of  $B_z$ .

The calculation of the drift is (apart from straightforward relativistic extensions) standard<sup>10</sup>. We assume that any gyro-motion is lost by synchrotron radiation near the light cylinder. Drifts involving  $v_{||}$  turn out to be negligible because of its  $r^{-2}$  dependence. (Thus the motion is eventually independent of initial conditions which appear through  $\mathcal{E}$ .) The dominant drift is

$$v_\perp = \frac{m}{q} \left\{ \mathbf{B} \times d(\gamma \mathbf{U}_E)/dt \right\} / B^2 \quad (10)$$

evaluated along the zero order trajectory. The corresponding corrected  $\gamma$  reduces eventually to

$$\gamma \approx F(r/r_1) + \delta G(r/r_1)^2 \quad (11)$$

where  $G$  is a second function of direction obtainable from a given force-free model, and  $\delta$  is the small number

$$\delta = \Omega/\omega_{BL} \quad (12)$$

where  $\omega_{BL}$  is a measure of the non-relativistic gyro-frequency at the light radius. For the Crab pulsar,  $\delta$  is of order  $10^{-11}$  for electrons and  $10^{-8}$  for protons.

The drift approximation will fail when the two terms in (11) are comparable. We can only speculate on what occurs beyond this but it seems likely that gyro-motion will appear<sup>1</sup>, and that there will be no further energisation. We assume that this is the case. In a given direction there will be a maximum value  $\gamma_M$ , and corresponding energy  $K_M$ , at a distance  $r_M$  where

$$r_M = R r_1/\delta, \quad (13)$$

$$K_M/mc^2 = \gamma_M = \Gamma/\delta,$$

where, roughly,  $R \approx F/|G|$  and  $\Gamma \approx 2F^2/|G|$ . We assume that the field falls as  $r^{-3}$  from the star to the light radius, thence as  $r^{-1}$ . If the stellar radius and field strength are  $R_s \times 10^8 K_M$ , and  $B_s \times 10^8 T$ , respectively, then

$$r_M/R = e\Omega R_s^3 B_s/mc^2 = 6 \times 10^{-8} \Omega R_s^3 B_s \text{ pc (electrons)}$$

$$= 3 \times 10^{-6} \Omega R_s^3 B_s \text{ pc (protons)} \quad (14)$$

$$K_M/\Gamma = e\Omega^2 R_s^3 B_s/c = 4 \times 10^{11} \Omega^2 R_s^3 B_s \text{ V}$$

$K_M$  has the same order as the  $\Phi_M$  of Goldreich and Julian<sup>1</sup>, and is about  $2 \times 10^{16}$  V for the Crab pulsar. The field at  $r_M$  is such that the non-relativistic gyro-frequency is comparable with  $\Omega$ . This is of course expected where the drift approximation breaks down. It is much larger than any possible interstellar field for protons, but for slower pulsar periods of order 1 s, it is of order  $10^{-10}$  T for electrons. Electrons therefore, may be lost from the system before attaining their maximum energy. The distance  $r_M$  would seem to be well within the surrounding nebula of any pulsar.

A rough estimate of the flux of energy from the system carried by the particles yields a figure comparable to the dipole radiation rate. Again then, it is clear that the approximations break down at  $r_M$ , and that a self-consistent approach must replace the force-free approximation at such distances.

Finally, the loss of energy  $\Delta K$  due to radiation reaction during the motion is negligible. This is basically because particles are travelling at the speed of light in straight lines. Using standard formulae<sup>11</sup> it is readily shown that the ratio  $\Delta K/K_M$  is of order  $\Omega t_r$ , where  $t_r$  is the classical radiation time scale  $10^{-23}$  s (electrons). Thus  $\Delta K$  is completely negligible.

Further details of the process, and the breakdown of the force-free approximation will appear elsewhere.

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## Redshift effect on the timescale of quasar radio variability

It has been suggested (ref. 1 and McCrea (I.A.U. Symposium No. 63, 1972)) that the radio or optical variability of quasars should be related to their redshifts. If the latter originate from the Doppler effect, or are gravitational, the observed fluctuation rate of the radio or optical emission should also be redshifted, that is, slowed down, in proportion to  $1+z$ , where  $z$  is the usual redshift factor. Medd *et al.*, measuring quasar flux densities with the Algonquin telescope, attempted to detect this effect by plotting the frequency of maxima occurrence in the flux-density curves against  $z$  but obtained an inconclusive result. Scargle<sup>2</sup> also analysed the Algonquin observations, using the half-width of the autocorrelation function of the flux-density curve as a measure of fluctuation rate. Again, the results show considerable dispersion, but Scargle argues that regression analysis supports the expected relationship. We present here a preliminary version of an analysis based on the power spectrum of the flux-density curves, which establishes the expected result.

Our analysis was restricted to the  $\lambda 2.8$  cm measurements (ref. 1 and B. H. Andrew and G. A. Harvey, personal communication) of the 33 identified quasars in the Algonquin sample for which redshifts were available. The data covered periods of 74–110 months between 1966.5 and 1975.7, a month being defined as 1/12 yr. The record lengths are variable, therefore; moreover, they contain gaps and the observations are not equally spaced in time.

The data were interpolated to produce equally-spaced samples at 1-month intervals by a weighted linear interpolation (convolution); the intervening function was triangular, with a base of 6 months. (This has a significant filtering effect on the power spectrum which was subsequently corrected.) Where the gaps were too wide to use this function, the data were interpolated linearly. The d.c. component of the record, and any linear trend, were removed by fitting a straight line in a least-mean-squares sense. A cosine taper was applied to 10% of the record length at each end, and all records were converted to a uniform length of 128 months by adding zeros at the end. The resulting records are 128 samples long, appropriate for applying the Fast Fourier Transform. The transformation results in spectra which are tabulated at intervals of 1/128 = 0.00781 cycle per month. The spectral amplitudes were then squared to arrive at the power spectra of the flux-density curves.

A typical spectrum is shown in Fig. 1a. At frequencies  $< 0.01$  cycle per month, a finite record length coupled with the absence of d.c. power biases the first three points downwards. At high frequencies the spectrum consists only of noise, representing the original measurement errors in the flux densities. Between these extremes is a regime where  $\ln P$  falls approximately linearly with frequency, shown more clearly in Fig. 1b. All 33 quasars have a similar form of spectrum, though noise makes the similarity less

clear-cut for those which vary weakly in comparison with the flux measurement errors. Attention was therefore restricted to those 13 quasars for which the s.d. of the time-series record exceeded that of the quoted measurement errors<sup>1</sup> by a factor of 1.7, which corresponds to a convenient break in the distribution of s.d. ratios.

If the variability spectrum of a given quasar could be measured for a redshift  $z = 0$  then at a finite redshift  $z$ , the effect on the spectrum would be to steepen the slope  $d(\ln P)/df$  by the factor  $1+z$ . A plot of slope against  $1+z$  for different quasars might also be expected to show this relationship, provided the dispersion in variability rate from one quasar to another is not so strong as to obscure it.

For each quasar, the slope was measured by computing the average values  $P_1$  and  $P_2$  of  $P$  over the frequency ranges 0.0117–0.0508, and 0.0508–0.0898 cycles per month, and forming the quantity  $(\ln P_1 - \ln P_2)/0.0391$ . The averaging ranges were chosen as a compromise between a wide range, which would give a good estimate of the slope, and the necessity to restrict the measurement to that part of the spectrum having a high signal-to-noise ratio. The results are plotted in Fig. 2. There is clearly evidence for a correlation in the expected sense.

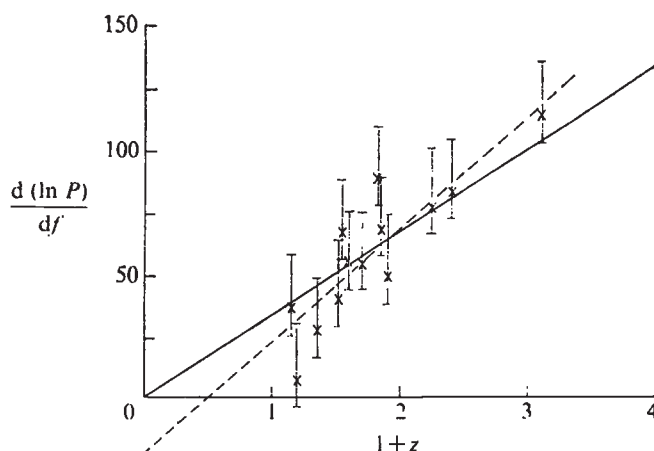


Fig. 2 Plot of  $d(\ln P)/df$  against  $(1+z)$ . The full line is a regression of  $y$  on  $x$  which is constrained to pass through the origin. The dashed line is not so constrained. The  $1\sigma$  confidence bars are derived from the  $\chi^2$  distribution of the spectral estimates.

Assuming that the time-series are random and not deterministic, and that they are noise-free, spectral estimates will follow a  $\chi^2$  distribution. Since the data were selected to have a high signal-to-noise ratio, this will be very nearly correct. Confidence limits of  $1\sigma$  have been computed (Fig. 2). The assumption of randomness is crudely verified *post facto* as the  $\chi^2$  estimates seem to be consistent with the observed scatter in the spectra.

Figure 2 shows a correlation in the expected sense between the spectral slope and the redshift, and the value of the correlation coefficient is computed to be 0.88. It is, however, difficult to set a proper confidence limit on this result, because the usual tests are based on a normal distribution of errors in the data, which is not the case here. Nevertheless, we have assumed normality, to set a rough confidence limit on the result, an assumption which finds crude justification in the central limit theorem<sup>3</sup>. The correlation coefficient is then significant at a level much lower than 1%.

Although the correlation is significant, the expected proportionality between spectral slope and  $(1+z)$  should cause the regression line to pass close to the origin. In fact, its intercept on the  $y$ -axis is  $-26.6$ , but the significance of this departure is only 7% (again assuming normality) so the fact that the regression line does not pass through the origin is not very significant. The regression of  $y$  on  $x$  with the line constrained to go through the origin is shown in Fig. 2.

Our establishment of the expected correlation between the observed rate of radio variability and redshift implies that whatever causes the redshift of the optical spectral lines must also slow down the observed variability rate. Because the unshifted variability rate is not known *a priori* (unlike the frequency of optical spectral lines), we cannot establish that the radio variability is subject to the same value of  $(1+z)$ . Our data would be consistent, for instance, with an interpretation in which the radio emission were subject to exactly half the optical value of  $(1+z)$ . If the redshift is supposed to have a Doppler (or a gravitational) origin, our data require that the optical and radio emission arise in regions with equal or related recessional velocities (or gravitational potentials).

An interesting inference from Fig. 2 is that the intrinsic spread of quasar variability rate (at a given redshift) is low, at most of the order of 3, and presumably lower if the effects of the  $\chi^2$  variance were reduced, by accumulating longer records. This suggests that variability rate may be a useful parameter for cosmological investigations, assuming the redshift is cosmological, and could be used, for instance, to measure redshift, as a function of cosmological epoch.

The exponential power-spectrum of quasar variability can be useful in considering the astrophysics of these objects. If the form of the spectrum is  $P = Ae^{-kf}$ , then the mean value of  $k$

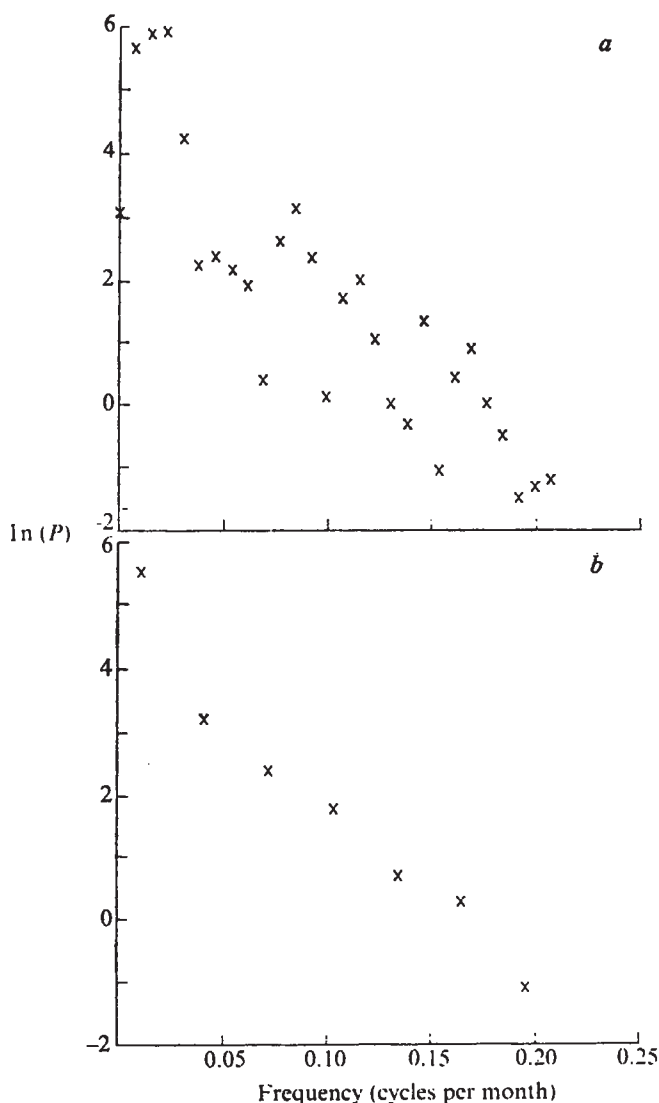


Fig. 1 Power spectra of the radio flux variations of CTA 26, corrected for the filtering effects of the convolving function. The ordinates are the natural logarithms of the power spectral density  $P$ , in units of  $\text{Jy}^2 (\text{cycles per month})^{-1}$ . *a*, raw spectrum at a resolution of 0.0078 cycles per month. *b*, spectrum smoothed by averaging in groups of four points.



can be deduced from Fig. 2 using the value of the ordinate of the full regression line for  $z = 0$ . We obtain  $k = 33.3$  (cycles per month) $^{-1}$ , implying that most of the variations take place at frequencies  $< k^{-1} = 0.03$  cycle per month.

Further work clearly depends on acquiring more data, and on minimising the effects of errors on the present data. The number of points in Fig. 2 could be more than doubled if the 20 weakly-variable quasars in our original sample could be used.

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## Whistlers recorded at Varanasi

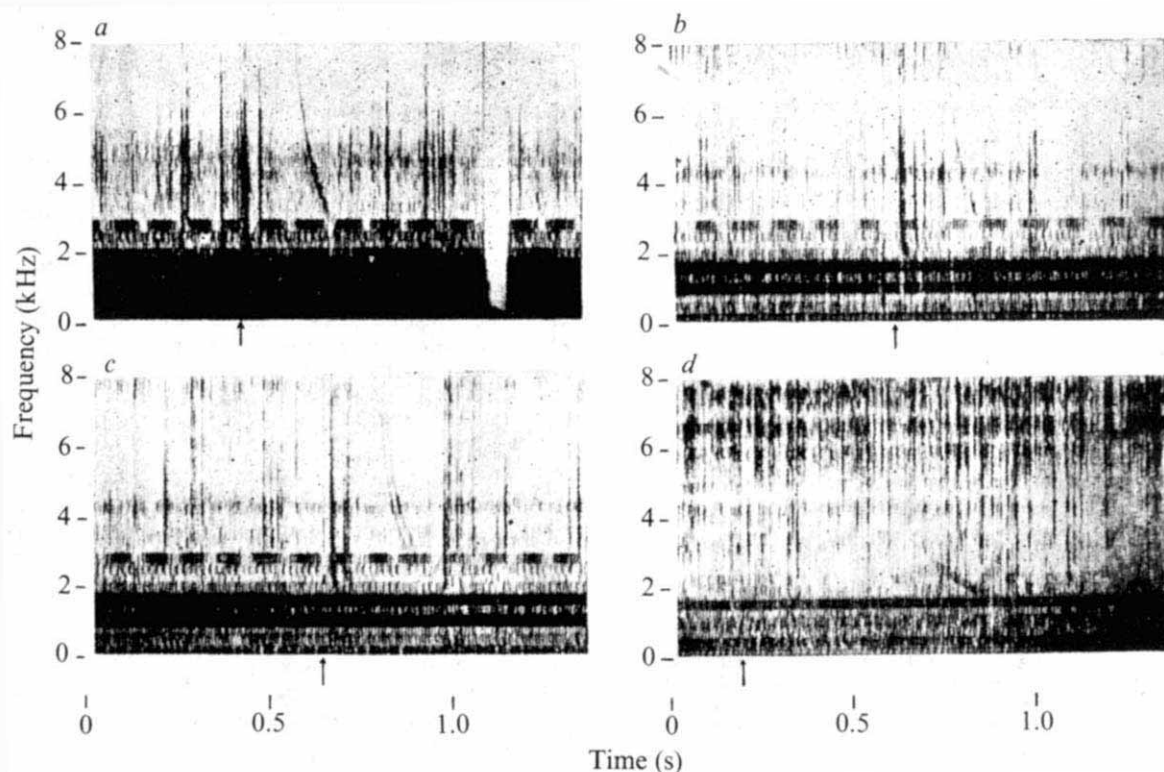
THE study of low-latitude whistlers dates back to the pioneering work of Japanese scientists. The whistlers were first recorded at Toyokawa (geomag. lat.,  $24.5^\circ\text{N}$ )<sup>1</sup>, and later efforts were made to record them at lower latitudes. The first successful records of whistlers in India were obtained at Gulmarg (geomag. lat.,  $24^\circ 10'\text{N}$ ) by Somayajulu *et al.*<sup>2</sup>. Recording was later carried out at Nainital (geomag. lat.,  $19^\circ 1'\text{N}$ ) and whistlers with comparatively lower dispersion and reduced rate of occurrence were reported<sup>3–5</sup>.

Unsuccessful efforts were also made to record whistlers at Varanasi (geomag. lat.,  $15^\circ 6'\text{N}$ ). Analysis of the low latitude whistler records by Rao *et al.*<sup>6</sup> showed that the low-latitude whistler cut-off lies around  $15^\circ$  latitude, and apparently provided the reason for the non-recording of whistlers at Varanasi. Using an improved system, we have succeeded in recording whistler activity at Varanasi, and we describe here the categories of sonograms recorded in April, 1976.

It is well known that the global thunderstorm activity is at a maximum at the temperate latitude and decreases with increasing latitudes<sup>7</sup>. The parameters or mechanisms controlling the guidance of thunderstorm-generated low-frequency waves are less effective at low latitude, however, which seems to reduce the occurrence of whistler activity. The combination of these two controlling factors maximises the rate of occurrence around  $50^\circ$  geomagnetic latitude and results into a low-latitude whistler rate cut-off. It is obvious that the sporadic redistribution of thunderstorm activity and so-called enhancement factor controlling the guidance of whistlers at times changes the latitude of maximum occurrence rate of whistlers and shifts the low-latitude cutoff to still lower latitudes.

The interpretation of such a low-latitude cutoff seemed artificial and efforts to record whistlers at Varanasi continued, using an improved antenna system and preamplifier. The recorded sonograms (Fig. 1) show much noise with very distinct causative atmospherics. The whistler activity at Varanasi showed highest occurrence rate during the months of February–April, 1976, and generally occurred in the post-midnight hours. They can be classified into four different categories. Figure 1a shows a single trace short whistler with distinct causative atmospherics: the dispersion is  $12\text{ s}^{1/2}$ . Figure 1b shows a feeble trace of banded whistler with almost similar dispersion features. The bands at lower frequencies are very clear, but are not distinct at higher frequencies. Figure 1c depicts two causative atmospherics

**Fig. 1** Sonogram of whistlers recorded at Varanasi. *a*, 0026, 7 April 1976, short whistler; *b*, 0106, 4 April 1976, banded whistler; *c*, 0039, 7 April 1976, twin whistler; *d*, 2242, 4 April 1976, whistler with high dispersion. A model UHER 4400 Report stereo tape recorder provided the continuously recorded sonograms.



and gives an initial impression of multiflash, but dispersion analysis showed this to be a short twin whistler. Multiflash whistlers have not yet been recorded at this station. The dispersion feature is almost similar to that shown in Figs 1a and b. A curious but frequent trace is shown in Fig. 1d, where only a part of the trace in the low-frequency region (2–4 kHz) is seen and the high-frequency part of the trace does not appear. The extrapolation of the trace and the location of causative atmospherics shows a comparatively large dispersion of  $25 \text{ s}^{1/2}$ . The non-appearance of high-frequency part in this sonogram associated with this higher dispersion is capable of revealing certain additional features of low-latitude propagation path. To our knowledge such records have not been reported either at high- or low-latitude stations. The non-appearance of the trace at frequencies higher than 4 kHz is related either to the enhanced absorption or to poor guidance of whistlers. Examination of the sonograms shows that such traces are often seen before or after the formation of banded whistlers (Fig. 1b and d). It therefore seems less probable that the enhanced average absorption of the propagation path is a likely cause of absence of high-frequency trace of the sonogram. At the first glance the absence of high-frequency trace can be visualised as a particular case of banded whistlers which are believed to be some manifestation of shape, size and movement of ionospheric irregularities<sup>8</sup>. A detailed dispersion analysis of these sonograms is now being carried out to study various ionospheric and magnetospheric features.

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## Argon degassing models of Mars

ON July 20, 1976 Viking I lander made a soft landing on the martian surface. Atmospheric pressure recorded on the surface by the lander was 7.7 mbar and main constituents of the atmosphere were 95%  $\text{CO}_2$ , 2–3%  $\text{N}_2$ , 1–2% Ar and 0.3–0.4%  $\text{O}_2$  (ref. 1). The  $(^{40}\text{Ar}/^{36}\text{Ar})$  isotopic ratio was  $2,750 \pm 500$  as measured by a mass spectrometer<sup>1</sup>. This high isotopic ratio suggests an unique history for the evolution of the martian atmosphere. The possible mechanisms of argon degassing models of Mars are discussed here.

Since the decay of radioactive  $^{40}\text{K}$  produces  $^{40}\text{Ar}$ , the time variation of  $(^{40}\text{Ar}/^{36}\text{Ar})$  isotopic ratio in a closed system can be determined by two initial ratios,  $(^{40}\text{Ar}/^{36}\text{Ar})_0$  and  $(^{36}\text{Ar}/\text{K})_0$ . For the present case  $(^{40}\text{Ar}/^{36}\text{Ar})_0$  at the time of the planetary formation is considered to be very small

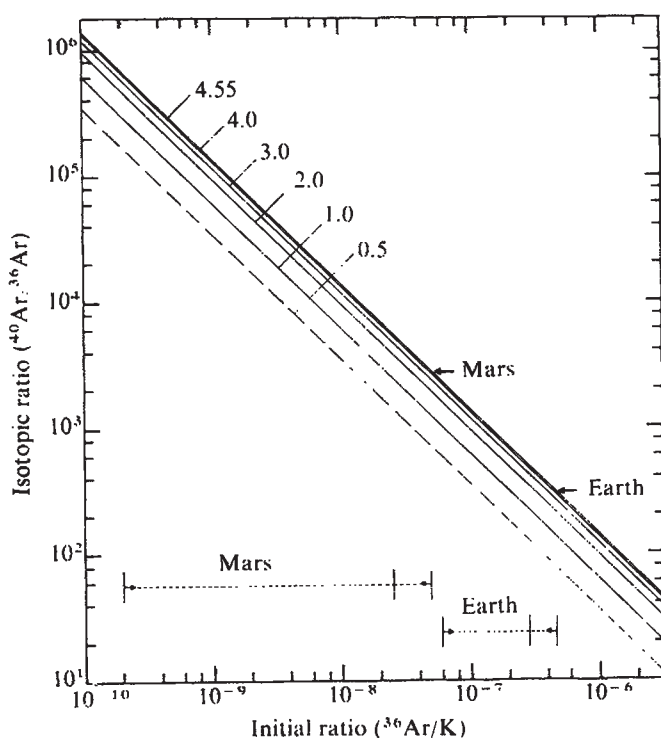


Fig. 1 Time variation of  $(^{40}\text{Ar}/^{36}\text{Ar})$  isotopic ratio in a closed system as a function of initial  $(^{36}\text{Ar}/\text{K})$  ratio. Two arrows indicate the isotopic ratios in terrestrial and martian atmospheres. Estimated ranges of the initial  $(^{36}\text{Ar}/\text{K})$  ratio for Earth and Mars are shown in the lower part.

( $\sim 10^{-4}$ ) from the theory of nucleosynthesis<sup>2</sup>. Hence the initial  $(^{36}\text{Ar}/\text{K})$  ratio uniquely determines the later  $(^{40}\text{Ar}/^{36}\text{Ar})$  ratio within the planet. The time variation of the isotopic ratio  $(^{40}\text{Ar}/^{36}\text{Ar})$  as a function of the initial  $(^{36}\text{Ar}/\text{K})_0$  ratio is shown in Fig. 1. Observed  $(^{40}\text{Ar}/^{36}\text{Ar})$  ratios in the terrestrial and the martian atmosphere are indicated by the arrows in the figure.

The present isotopic ratio  $(^{40}\text{Ar}/^{36}\text{Ar})$  in the atmosphere depends on the degassing history of Ar from the planetary interior, so these observed atmospheric values can not determine the initial  $(^{36}\text{Ar}/\text{K})_0$  ratio. The possible range of the ratio, however, can be inferred from the observed isotopic ratio. If we assume the atmospheric Ar is degassed fraction from the planetary interior, the  $(^{40}\text{Ar}/^{36}\text{Ar})$  ratio in the interior should be greater than the atmospheric value. This gives a maximum estimate for the initial  $(^{36}\text{Ar}/\text{K})_0$  ratio. For the Earth this value is about  $4.5 \times 10^{-7}$ . For the minimum estimate of  $(^{36}\text{Ar}/\text{K})_0$ , we can assume  $^{36}\text{Ar}$  has completely degassed to the atmosphere. This gives a value of  $6 \times 10^{-8}$  for the Earth with a chondritic mantle. The same argument suggests a possible range,  $2 \times 10^{-10} < (^{36}\text{Ar}/\text{K})_0 < 4.5 \times 10^{-8}$ , for Mars. These estimated possible ranges are shown in the lower part of Fig. 1.

Assumptions on the degassing model of Ar are needed to narrow the range. Turekian<sup>3</sup>, assuming a first-order rate process for Ar degassing, obtained the degassing rate constant from the abundance of  $^{40}\text{Ar}$  in the terrestrial atmosphere. Using his result and the atmospheric  $^{36}\text{Ar}$  abundance, the initial  $(^{36}\text{Ar}/\text{K})_0$  ratio is estimated to be about  $3 \times 10^{-7}$ . Although he assumed a chondritic Earth (K content 880 p.p.m.), the initial ratio is almost independent of the assumption of K content in the Earth. Ozima<sup>4</sup> concluded initial catastrophic and later continuous Ar degassing model from the suggested high  $(^{40}\text{Ar}/^{36}\text{Ar})$  ratio (more than 2,000) in the present mantle. This model requires a smaller  $(^{36}\text{Ar}/\text{K})$  ratio than the continuous degassing model. The



continuous degassing model for the martian atmosphere gives a value of  $3 \times 10^{-8}$  for the initial ( $^{36}\text{Ar}/\text{K}$ ) ratio.

This analysis of the Ar degassing model suggests a smaller ( $^{36}\text{Ar}/\text{K}$ )<sub>0</sub> ratio for Mars than for the Earth. This implies a higher content of K and/or a lower concentration of  $^{36}\text{Ar}$  in the whole of Mars than in the Earth. Physical adsorption is the only known quantitative explanation of planetary primordial rare gas in chondritic meteorites<sup>5</sup>. The size of the adsorbed fraction is very sensitive to temperature condition and increases as temperature decreases. As Mars is further from the Sun than the Earth, and considering the pre-planetary history of the solar nebula<sup>6</sup>, it is probable that Mars is at least as rich in original volatiles as the Earth. It seems likely that the K content of Mars is similar to that of normal chondrites<sup>7</sup> (900 p.p.m.). Even if we assume that the atmospheric Ar on Mars consists of very recent degassed fraction from the interior, the required K content of Mars is about 1,200 p.p.m. An even higher K content is necessary to explain the apparent stationary volcanic activity on the martian surface<sup>8</sup>.

To overcome this difficulty, we must assume that  $^{36}\text{Ar}$  (and probably other volatiles) escaped from the martian region during the geological history of Mars. This escaping mechanism should operate in the early stage of the evolution of Mars, where ( $^{36}\text{Ar}$ ) is more abundant than ( $^{40}\text{Ar}$ ). The explanation of the chemical composition of the planets by the condensation theory<sup>9</sup>, requires that the solar nebula escapes from the inner planetary region at the early stage of the solar evolution. This process may contribute to remove the initially degassed volatiles from Mars. Although the above discussion is based on the atmospheric content of Ar, the degassing history of volatiles closely related to the differentiation process in the planet, which can be inferred from petrological investigations of surface rocks as studied for the Moon<sup>3</sup>.

If such investigation reveals an early large-scale differentiation of Mars, the above conclusion would be confirmed. I thank Professor M. Ozima for valuable discussions and encouragement.

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## Conductivity dispersion in single-crystal $\beta$ -alumina electrolyte

WE report data which demonstrate the existence of a conductivity dispersion in single-crystal  $\beta$ -alumina and, we believe, for the first time, a distribution of conductivity relaxation times in any single-crystal ionic conductor. A cylindrical boule of high quality Na  $\beta$ -alumina grown at Union Carbide was supplied to us by Roditi International Corporation. Figure 1 shows the frequency-dependent conductivity of this crystal in the range  $10^2$ – $10^7$  Hz, measured at various subambient temperatures (for details of the apparatus see refs 1 and 2). At normal temperatures, electrode effects produce an apparent dispersion in conductivity. At these lower temperatures, however, the dispersion seems to be an intrinsic property of the bulk material since it starts at frequencies which are related to the level of the d.c. conductivity ( $f = \sigma/2\pi\epsilon$ )<sup>1,3</sup> as indicated by

arrows in (Fig. 1). This behaviour closely resembles the dielectric behaviour which has been studied in silicate and other glasses<sup>3,4</sup>. The dispersions in conductivity cannot be accounted for in terms of the conventional (Debye) theories of dielectric loss; indeed, the departures from Debye behaviour are probably more marked in single-crystal  $\beta$ -alumina than in many vitreous electrolytes.

This result can be interpreted in terms of a broad distribution of conductivity relaxation times, which could plausibly be related to the random distribution of sodium ions over distinct crystallographic sites within the conduction planes (as revealed for example by recent X-ray and neutron diffraction studies)<sup>5,6</sup>. These studies have shown that at  $\approx 90$  K, sodium ions are localised at three distinct sites, but with increasing temperature there is increasing cation mobility, and by 620 K the disorder resembles that of a two-dimensional fluid. The details of this picture have been confirmed by NMR spectroscopy<sup>7</sup>, but of

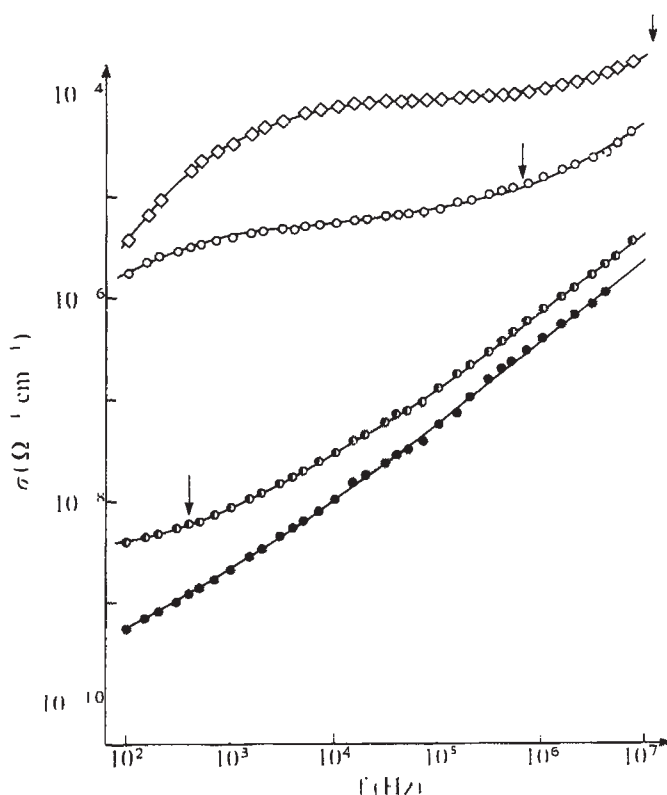


Fig. 1 Frequency-dependent conductivity of single-crystal  $\beta$ -alumina at 77 K (●), 87 K (○), 113 K (□) and 143 K (◇).

special relevance to this discussion, it should be noted that the existence of a 'wide distribution in ion jump frequencies' was postulated, to account for certain aspects of the data. The conductance dispersion which we have observed would then follow directly from a model involving sequential hopping of ions over the different crystallographic sites.

It is also possible, however, to place a different interpretation on these data. In Fig. 1, it is apparent that at low temperatures, the high-frequency or a.c. conductivities fit a 'universal law' which has been described by Jonscher<sup>8,9</sup>. In the region of conductance dispersion, the a.c. conductance  $\sigma(\omega)$  is given by:

$$\sigma(\omega) \propto A\omega^n \quad (1)$$

where  $n = 0.78$  at 87 K, and is somewhat insensitive to changes in temperature. This 'law' is obeyed by a wide range of materials, and an interpretation has been given by Jonscher in terms of a 'screened charge model' where the ratio of energy stored to energy lost per cycle is independent of frequency. On the basis of this theory the conductivity dispersion in  $\beta$ -alumina need not be related to a microscopic distribution of ionic jump



frequencies, but could be understood in terms of an interionic 'drag effect' which gradually relaxes with increase in frequency. (It is tempting to relate this approach to a previous treatment of dielectric loss in vitreous materials which invoked the Debye-Falkenhagen effect<sup>11</sup>.)

It is difficult to say that either of these explanations is 'right' and the other 'wrong'. Thus, according to the Jonscher model, highly 'lossy' electrolytes, such as  $\beta$ -alumina, need a high concentration of mobile ions to give effective charge-screening, but these are precisely the electrolytes which have a strong tendency to cation disorder and hence are likely to exhibit a wide spread in ionic jump frequencies<sup>12</sup>. The main point which emerges from the above discussion is a need for further detailed comparisons of structural, spectroscopic and electrical data, and hence the need for work with crystalline rather than vitreous systems.  $\beta$ -alumina is in many ways a model system, since measurements can be made over a wide range of d.c. conductivities, and extensive cation substitution is possible<sup>13,14</sup>.

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## The behaviour of long molecules diffusing in solid polyethylene

SEMI-CRYSTALLINE polymers, according to the current two-phase model, consist of an impermeable, denser crystalline phase (the lamellae) and a permeable, less dense amorphous phase (the interlamellar regions)<sup>1</sup>. All permeation and transport properties occur predominantly in the latter, and are a function of the overall amorphous fraction within the bulk and the geometry of the crystallites embedded in the amorphous phase. For linear polyethylene cooled at various rates from the melt, the diffusion coefficient  $D$  of gaseous diffusants varies<sup>2,3</sup> according to

$$D \propto (1-x), \quad (1)$$

where  $x$  is the corresponding crystalline volume fraction, and  $(1-x)$  the amorphosity. The diffusional behaviour of long chain diffusants, however, may be very different. For example, it has been suggested<sup>4</sup> that the transport of long chain molecules through the disordered interlamellar regions of polythene will be greatly influenced by the presence of interlamellar tie-molecules and entanglements. These are relatively immobile features in the disordered region which can obstruct the movement of long chain molecules, but not that of smaller (for example, gaseous) diffusants if their size is less than the separation between these features. An extension of de Gennes' ideas on reptation<sup>5</sup> gives a semiquantitative relation for the diffusion coefficient of long molecules in polyethylene: long

molecules should diffuse faster in slowly cooled than in quenched polyethylene, in spite of the higher disordered fraction in the latter and in marked contrast to the behaviour of gaseous or light vapour diffusants, because rapid cooling results in a greater profusion of fixed tie-molecules<sup>6</sup>, which constrain long diffusants but have little effect on small ones. We report here results which support this model.

We measured  $D$  for two linear diffusants of the form  $n$   $(\text{CH}_2)_n\text{X}$ , X being a suitable label and  $n \approx 25$  or 45. The matrix was linear polyethylene cooled at two widely differing rates from the melt. The measuring technique was one we have recently developed, based on infrared microdensitometry<sup>7</sup>, and involves setting up a known concentration distribution of diffusant within the host matrix, allowing it to broaden for a known time  $t$  at the required temperature, then measuring the resulting diffusion-broadened concentration profile.  $D$  can be evaluated knowing the shapes of the two profiles as well as  $t$ : Fig. 1 shows experimental profiles at the beginning and end of a typical diffusion run: the calculated curves (with the appropriate value of  $D$ ) when superimposed on the experimental ones, show a good fit.

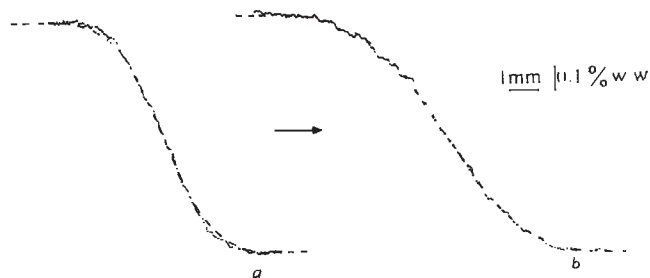


Fig. 1 Concentration-distance profiles as measured (—) at the beginning (a) and end (b) of a typical diffusion measurement. ----, calculated shapes as evaluated with the appropriate value of  $D$ . ( $t = 1.29 \times 10^6$  s at 91 °C; the diffusant is a linear molecule of form  $n$   $(\text{CH}_2)_n\text{X}$ , X being an ester group; the matrix is linear polyethylene cooled rapidly from the melt, and the scanning frequency corresponds to the ester group absorption peak at  $1729 \text{ cm}^{-1}$ .  $D$  evaluated as  $1.3 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$ .)

Table 1 summarises some of the results, giving  $D$  for the two diffusants in both rapidly and slowly cooled polyethylene matrix, and the corresponding values of the crystallinity  $x$  as derived from density measurements. In Table 2 we compare the prediction of the model (outlined above), which takes into account the topological constraints on the diffusants, with the experimental results (details of the calculations are given elsewhere<sup>8</sup>). The predictions of the simple two-phase relation, equation (1), are also shown.

Table 2 shows that agreement between observed and predicted ratios is fair to good. In particular, the model predicts correctly that  $D$  for the long diffusants used increases in the slowly cooled, more crystalline matrix—in contrast to equation (1).

The results provide support for the concept that long diffusants in semi-crystalline polymers may be constrained in a way which small diffusants are not: they suggest that studies involving such diffusants could yield information on the nature of the disordered phase in these polymers not otherwise available.

Table 1  $D$  (in units of  $10^{-10} \text{ cm}^2 \text{ s}^{-1}$ ) for diffusants of form  $n$   $(\text{CH}_2)_n\text{X}^*$  in linear polyethylene, at 84.8 °C

| No. of backbone units $n$        | Rapidly cooled matrix | Slowly cooled matrix |
|----------------------------------|-----------------------|----------------------|
| $n \approx 45$                   | $3.3 \pm 0.4$         | $6.5 \pm 0.7$        |
| $n \approx 25$                   | $9 \pm 1.8$           | $11 \pm 1.8$         |
| Crystalline volume fraction, $x$ | 0.751                 | 0.855                |

\*X is an ester group.

**Table 2** Calculated and observed values of  $D$  for diffusants of form  $n$   $(CH_2)_nX$  in linear polyethylene matrix at 84.8 °C(a) Variation of  $D$  with cooling rate of matrix

| No. of backbone units $N$ : | $D$ (slowly cooled matrix); $D$ (rapidly cooled matrix) |               | '2-phase',<br>$D \propto (1-x)$ |
|-----------------------------|---------------------------------------------------------|---------------|---------------------------------|
|                             | Calculated                                              | Observed      |                                 |
| $N \approx 25$              | 1.26                                                    | $1.2 \pm 0.3$ | 0.58                            |
| $N \approx 45$              | 1.52                                                    | $2.0 \pm 0.3$ | 0.58                            |

(b) Variation of  $D$  with diffusant length

| $\frac{D_{N=25}}{D_{N=15}}$ : | Rapidly cooled matrix |               | Slowly cooled matrix |               |
|-------------------------------|-----------------------|---------------|----------------------|---------------|
|                               | Calculated            | Observed      | Calculated           | Observed      |
|                               | 2.18                  | $2.7 \pm 0.5$ | 1.80                 | $1.7 \pm 0.3$ |

Detailed accounts of both theory<sup>4</sup> and experiment<sup>5</sup> are in press.

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## New transient effect observed in rock samples subjected to simulated upper crustal conditions

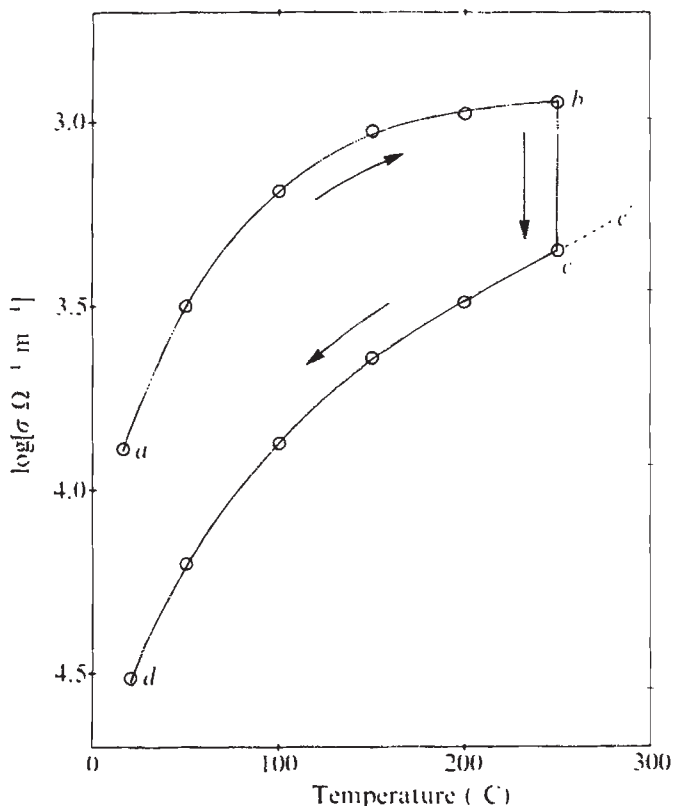
WHILE making laboratory measurements of the electrical conductivity ( $\sigma$ ) of rock samples subjected to temperatures, confining pressures, and pore fluid pressures thought to be appropriate to depths up to 15 km within the Earth, we have observed a hitherto unreported transient behaviour which is only revealed by the simultaneous application of pressure and temperature, and leads to an irreversible decrease in  $\sigma$  with time which could be due to compaction of the rock samples. We suggest that only after such a change has occurred is the condition of rocks collected near the Earth's surface more representative of those at depth in the crust.

Most of the rock samples used in this study are high-grade metamorphics from the Lewisian of NW Scotland, which have a very low porosity (< 1%). These were cored, cut and ground into cylinders of diameter 24.5 mm and length 10-20 mm, and saturated with a 0.5 or 0.05 M sodium chloride solution. Two stainless steel electrodes allowed the a.c. conductance (normally at a frequency of 1.5 kHz) between the sample faces to be measured using a Wayne Kerr B224 transformer bridge. Confining pressure acting on a rock within the Earth (due to the column of rocks above it) was simulated by hydrostatically pressurising the sample (up to 0.4 GPa (4 kbar)) in a pressure vessel. To prevent contamination from the oil pressure-medium the sample and electrodes were sheathed with a Teflon tube. It is believed that most rocks have sufficient strength to prevent their

pores shutting completely under such pressure<sup>1</sup>, and it is possible therefore for the pore fluid pressure to differ from the confining pressure. To allow for this, a hole passing through one electrode to the surface of the rock sample was connected to a pipe which passed out of the vessel, enabling the pore fluid pressure to be varied independently of the confining pressure. A furnace around the sample allowed temperatures up to 275 °C to be reached.

Because the bulk of the measured conductance in saturated rocks occurs through the solutions within the fine pore network, small changes in pore structure have a considerable effect on  $\sigma$  (refs 1, 2), and as  $\sigma$  can be measured accurately, it is probably the most sensitive property to use to investigate such small structural changes. Increasing the confining pressure closes up this pore structure, while increasing the pore fluid pressure has the opposite effect. It is reasonable therefore to assume a model in which the parameter that determines the overall pore structure, and therefore  $\sigma$ , is the pressure differential (known as the effective pressure) between confining and pore fluid pressure<sup>3,4</sup>. Most of our results at room temperature conformed to this model, with  $\sigma$  showing a steady decrease as the effective pressure was increased.

Figure 1 shows the typical behaviour observed when a sample at a fixed effective pressure was heated. From its room temperature value ( $a$ ),  $\sigma$  increased along the path  $a-b$  as the temperature was increased. We found, however, that if the temperature was held steady at a high enough temperature ( $b$ ),  $\sigma$  fell over a period of time to a lower value ( $c$ ). This drop  $b-c$  did not occur suddenly at any specific temperature, and a slight drop has even been observed at temperatures as low as 130 °C, although at a very slow rate. Increasing the temperature has the effect of accelerating the rate, and samples were normally heated to around 200 °C or above, to enable us to observe a drop in a reasonable period of time. Figure 2a shows the time dependence



**Fig. 1** Irreversible change in conductivity during the initial temperature cycle of a sample of gneiss saturated with 0.5 M NaCl solution and subjected to an effective pressure of 0.2 GPa (2 kbar).

of  $\sigma$  in one sample during the period of the drop. On cooling, the path  $c-d$  was followed. With any further increase or decrease in temperature,  $\sigma$  remained on the curve  $d-c$ , even when the temperature was increased beyond the point  $c$  to  $c'$ , indicating that the rock had suffered some irreversible change. Although the rate of the drop varied from sample to sample, the general cycle  $abcd$  was observed in all saturated samples investigated. The feature does not seem to be confined to the very low porosity rock type used, since samples of basalt (2% porosity) and pyrophyllite (7% porosity) also showed a similar drop. Only in a sample pre-baked to remove any natural pore fluids was there no discernible drop in  $\sigma$  at high temperatures. Spurious effects, such as conduction paths between the rock and the Teflon sleeve, were discounted after making measurements with non-porous plugs (Teflon or sapphire) replacing the rock sample.

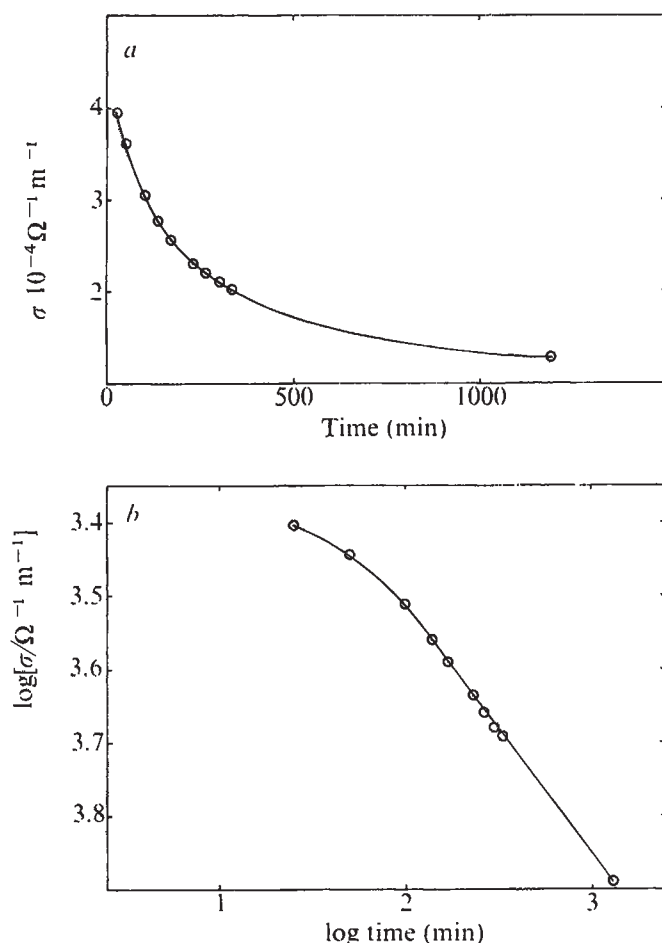


Fig. 2 Time dependence of the conductivity for a sample of granite gneiss (saturated with a 0.5 M NaCl solution) when subjected to an effective pressure of 0.35 GPa (3.5 kbar) at a temperature of 190 °C.

We believe a possible explanation for this behaviour is that in its initial state  $a$ , the sample contains many cracks and flaws associated with near-surface conditions such as weathering and unloading (and possibly with rock sampling and preparation)<sup>6</sup>. At room temperature, the strength of a rock is sufficient to prevent many of these cracks closing under pressure, but at elevated temperatures, the strength of a rock decreases, allowing more of the initial cracks to close and leaving the rock in a more compacted state. Figure 2b shows that apart from the initial period of the drop (where there is uncertainty in the

precise onset), the variation of  $\sigma$  with time ( $t$ ) may be expressed by the equation:

$$\log \sigma = A + B \log t,$$

where  $A$  and  $B$  are constants dependent on rock type, and the temperature and pressure conditions.

It has often been observed in transient creep measurements on rocks subjected to differential stress, that strain ( $\epsilon$ ) is also proportional to  $\log t$  (refs 6, 7). If it could be shown, therefore, that a linear relationship exists between  $\log \sigma$  and  $\epsilon$ , it is possible that the drop we observe in  $\log \sigma$ , when held at high pressure and temperature, may also be due to creep behaviour. Such a relationship might be argued as follows. In saturated rocks, it is found experimentally<sup>1,2</sup> that at moderate pressure ( $p$ ),

$$\log \sigma \propto p.$$

Furthermore, from strain measurements made under such pressures<sup>1</sup>,

$$\epsilon \propto p.$$

Thus, it seems reasonable to assume that for any strain (whether elastic or inelastic)

$$\log \sigma \propto \epsilon.$$

If this is the case (that is, that this transient behaviour is a sensitive indicator of a creep process), then presumably it is important to simulate this effect by thermal cycling of a rock sample under pressure before truly meaningful measurements of various physical properties of rocks can be made under laboratory conditions. To investigate this possibility, we are presently making simultaneous measurements of acoustic velocities and electrical conductivity in rocks, under the same conditions as described earlier, to examine whether the transient behaviour in  $\sigma$  reported here may also be observed in acoustic velocity measurements.

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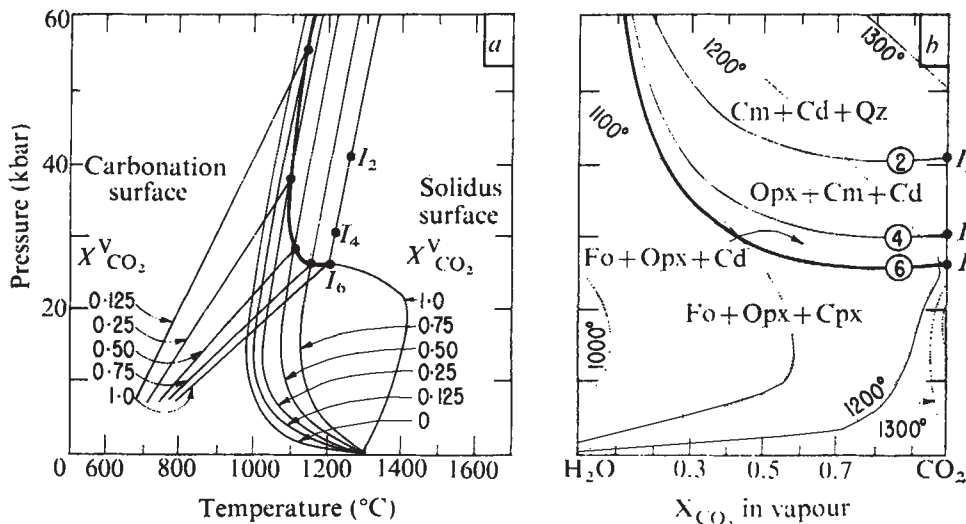
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## Peridotite-CO<sub>2</sub>-H<sub>2</sub>O, and carbonatitic liquids in the upper asthenosphere

THE recent discovery that CO<sub>2</sub> at upper mantle pressures has a dramatic effect on the melting temperature of peridotite (because the silicate assemblage becomes carbonated), and on the composition of near-solidus liquid, has petrological and geophysical implications that have been explored extensively. The influence of H<sub>2</sub>O has received only passing reference, probably because it was tacitly assumed that this would modify the carbonate-dominated melting relationships significantly. I describe here construction of a schematic diagram for the system peridotite-CO<sub>2</sub>-H<sub>2</sub>O which shows that the carbonate can persist even in the





**Fig. 1** Schematic phase diagram for the system peridotite-H<sub>2</sub>O-CO<sub>2</sub>, assuming a simple peridotite composition, Fo 65%, Opx 23%, Cpx 12%. This illustrates the geometry for intersection of a divariant subsolidus carbonation reaction with the divariant solidus surface for melting in the presence of CO<sub>2</sub>-H<sub>2</sub>O vapours. It represents a compromise among several sets of experimental data, and precise pressures and temperatures need experimental determination (see ref. 21 for modifications of data from refs 5, 6, 10, 12, 13, 22). *a*, *P-T* projection. The univariant carbonation reaction extending to lower pressures and temperatures

from  $I_6$  is: (6)  $\text{Cpx} + \text{Fo} + \text{CO}_2 = \text{Opx} + \text{Cd}$  (calcite dolomite)<sup>1-6</sup>. In the presence of CO<sub>2</sub>-H<sub>2</sub>O vapours this reaction becomes a divariant surface extending to higher pressures and lower temperatures; it is mapped by contours for constant vapour-phase composition. Curves for the beginning of melting of peridotite in the presence of water<sup>22</sup> and CO<sub>2</sub><sup>1-6</sup> are connected by a curved surface, mapped in terms of contours for constant vapour-phase composition<sup>10</sup>. *b*, *P-X* projection ( $X$  is molar fraction). This shows the solidus surface, with estimated temperature contours. Vertical lines in this projection correspond to the contours in (*a*). The heavy line (6) corresponds to the intersection line of the two surfaces illustrated in (*a*). The lines (4) and (2) refer to other carbonation reactions<sup>1-4</sup> that are not encountered for reasonable mantle peridotite compositions, with low CO<sub>2</sub> contents. Mantle vapour-phase compositions are buffered to remain on the divariant carbonation surface of (*a*). Therefore, at pressures (depths) greater than  $I_6$ , carbonate is stable in peridotite with total CO<sub>2</sub>/H<sub>2</sub>O ratios greater, at a given pressure, than the heavy line (6) in (*b*); similarly, vapour-phase compositions within the heavy line of (*b*) do not exist in the mantle at the pressures indicated. Abbreviations: Cm, magnesite solid solution; Qz, SiO<sub>2</sub> polymorphs.

presence of vapours with high H<sub>2</sub>O/CO<sub>2</sub>. Figure 1 shows that starting with peridotite-H<sub>2</sub>O at high pressures, a small addition of CO<sub>2</sub> is sufficient for the generation of carbonate. This leads to the unexpected conclusion that many of the applications deduced for the melting of carbonated peridotite may remain valid with vapours enriched in H<sub>2</sub>O/CO<sub>2</sub>. In particular, the interstitial liquid in the upper part of the seismic low-velocity zone may be carbonatitic even with H<sub>2</sub>O present as a component.

Until recently, it was believed that the solubility of CO<sub>2</sub> in mantle magmas remained low as it does in crustal magmas<sup>1,2</sup>. Experimental studies in silicate-CO<sub>2</sub> and silicate-carbonate systems have shown that peridotite becomes carbonated by the reaction  $\text{Cpx} + \text{Fo} + \text{CO}_2 = \text{Opx} + \text{Cd}$  at high pressures<sup>3-6</sup> (Fo, forsterite; Cd, calcite dolomite; Opx, orthopyroxene; Cpx, clinopyroxene), and this causes a remarkable increase in the CO<sub>2</sub>-solubility of near-solidus liquids<sup>4,7,8</sup>. The composition of the liquid produced by melting of the silicate-carbonate assemblage along the peridotite-CO<sub>2</sub> solidus rising from  $I_6$  in Fig. 1*a* is 'carbonatitic' according to Wyllie and Huang<sup>4,5,7</sup>, with CO<sub>2</sub> solubility approaching 40%, and 'kimberlitic' according to Eggler<sup>6,8</sup>, with CO<sub>2</sub> solubility about 20%. Certainly, the CO<sub>2</sub> solubility is remarkably high, and the liquid composition is markedly different from that of normal basaltic magmas.

There is now convincing evidence that both H<sub>2</sub>O and CO<sub>2</sub> do exist in the upper mantle<sup>9-11</sup> and it has been shown experimentally that the proportion of CO<sub>2</sub>/H<sub>2</sub>O influences the SiO<sub>2</sub> content of near-solidus liquids generated from mantle peridotite<sup>10,12,13</sup>. Figure 1 illustrates the effect of H<sub>2</sub>O on the phase relationships for peridotite-CO<sub>2</sub>; the geometrical relationships are correct although specific values for pressure and temperature need experimental determination.

It is assumed that the peridotite composition is not suitable for the stabilisation of amphibole and phlogopite, so that we can examine the influence of carbonate alone. Consider a partially carbonated peridotite composed of  $\text{Fo} + \text{Opx} + \text{Cpx} + \text{Cd}$  in the presence of a small amount of

CO<sub>2</sub>-H<sub>2</sub>O vapour. This system must be situated on the divariant carbonation reaction surface and, at a given pressure and temperature, the vapour phase composition is given by the contour passing through the *P-T* point on the surface. With increase in temperature, decarbonation proceeds releasing CO<sub>2</sub> so that the CO<sub>2</sub>/H<sub>2</sub>O ratio in the vapour maintains the system on the surface. Similarly, carbonation occurs with increase in pressure, enriching the vapour in H<sub>2</sub>O/CO<sub>2</sub>. The principle is well established in metamorphic systems<sup>14,15</sup>; the vapour phase composition is buffered by the mineralogy.

The system can leave the surface for divariant reaction (6 in Fig. 1) only if carbonation or decarbonation are completed. The first alternative requires complete reaction of 12% clinopyroxene, which is most unlikely for any reasonable CO<sub>2</sub> content in mantle peridotite; therefore, the system cannot enter the high-pressure volume for complete carbonation in Fig. 1*a*, which meets the solidus surface in the area at pressures above the heavy line in Fig. 1*b*.

At a given pressure above  $I_6$ , the vapour phase in partially carbonated peridotite at its solidus temperature is constrained to lie on the solid line (6) in Fig. 1*b* (compare its position in Fig. 1*a*). For peridotite without crystalline carbonate, the vapour phase at the solidus temperature may have any composition at pressures below  $I_6$ , but it is limited to the higher H<sub>2</sub>O/CO<sub>2</sub> ratios to the left of the heavy line (6) in Fig. 1*b*.

For the system peridotite-CO<sub>2</sub> at pressures above  $I_6$ , I am convinced that the near-solidus liquid is carbonatitic becoming kimberlitic with increasing temperature<sup>4,5,7</sup> (contrast Eggler). From the scanty data available in silicate-carbonate-CO<sub>2</sub>-H<sub>2</sub>O systems<sup>16-18</sup>, I anticipate that the liquid composition at the solidus is dominated by the silicate-carbonate assemblage; any H<sub>2</sub>O in the vapour dissolves in this liquid, with relatively little influence on the liquid composition. Accordingly, the liquid produced from the peridotite-carbonate assemblage could remain 'carbonatitic', even in the presence of H<sub>2</sub>O/CO<sub>2</sub>-enriched vapours. At pressures below  $I_6$ , near-solidus liquids become more siliceous with increasing H<sub>2</sub>O/CO<sub>2</sub><sup>10,13</sup>. At pressures above

$I_0$ , this is true also for the range of vapours coexisting with uncarbonated peridotite. Using the schematic phase diagram of Fig. 1 as a guide, we can reach the following conclusions.

For a partially carbonated mantle peridotite of fixed composition in thermal equilibrium, it can be determined from Fig. 1 that the amount of carbonate in the lithosphere decreases with depth, and the ratio of  $\text{CO}_2/\text{H}_2\text{O}$  increases in the vapour phase. If a portion of the mantle rises from the geotherm under adiabatic conditions, the amount of carbonate decreases, and the ratio of  $\text{CO}_2/\text{H}_2\text{O}$  in the vapour increases as the peridotite rises to shallower levels.

For a partially carbonated mantle peridotite, coexisting vapours have fairly high ratios of  $\text{H}_2\text{O}/\text{CO}_2$  (Fig. 1b). Incipient melting begins with the generation of carbonatitic liquid. The liquid at the top of the seismic low-velocity zone may therefore be carbonatitic, with 10–15% dissolved silicates,  $\text{Ca}/\text{Mg} > 1$ , and probably enriched also in alkalis and a host of trace elements. At greater depths, this grades into liquids with kimberlitic affinities. Carbonatitic liquids are very fluid, and they would probably migrate upwards. Local concentrations, and intrusions into the overlying peridotite, could produce a layer of lithosphere enriched in crystalline carbonates.

This picture is consistent with a tentative proposal<sup>19</sup> that kimberlite magmas may be mixtures of silicate liquids from within the asthenosphere with carbonatitic liquids from near the top of the asthenosphere. It is also consistent with the suggestion<sup>20</sup> that carbonatite magmas could be the fluids involved in the fluidisation emplacement of some kimberlites. For mantle peridotite with compositions appropriate for the formation of phlogopite, vapour phase compositions must be buffered by subsolidus reactions between phlogopite and carbonate in the presence of  $\text{Fo} + \text{Opx} + \text{Cpx}$ , which remain to be determined<sup>21</sup>. Alternatively, all  $\text{CO}_2$  and  $\text{H}_2\text{O}$  may be stored in phlogopite and carbonate, with no free vapour phase.

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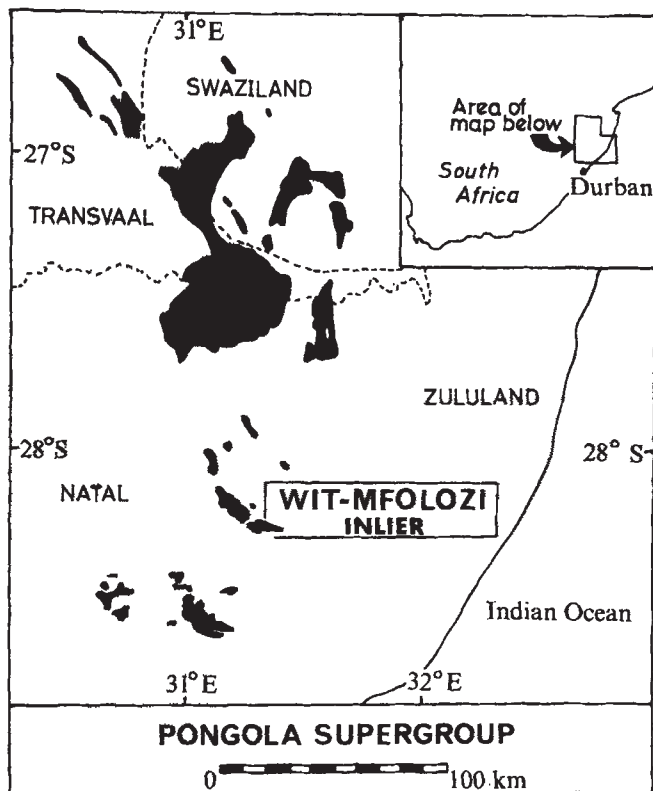


Fig. 1 The distribution of Pongola Supergroup exposures in the southeastern Transvaal, Swaziland, northern Natal and Zululand.

stratigraphic units; the lower 1,800-m thick Insuzi Group and the upper 700-m Mozaan Group<sup>1</sup>. Bedding and primary sedimentary structures are ubiquitously well preserved. Deformation is restricted to moderate cleaving of the argillaceous rocks. Evidence of regional metamorphism is restricted to incipient recrystallisation of the arenaceous and carbonate rocks; the lavas show evidence of deuteric alteration. Contact metamorphism is found where isolated diabase sills intrude the strata.

The Insuzi Group rests nonconformably<sup>2</sup> on crystalline basement rocks and comprises a repetitive sequence of quartz arenites and dolomites, with subordinate siltstone-mudstone units. The carbonates are of particular interest as no other recorded Insuzi Group exposures contain dolomites. They vary in thickness (1–30 m), and are laterally impersistent, forming units in the succession which are only of local importance. Amygdaloidal lavas occur near the base and at the top of the Insuzi Group. The siliciclastic sediments provide evidence for deposition in a tide dominated environment, as they contain sedimentary structures including lenticular, flaser and wavy bedding, mudclast conglomerates, herringbone cross lamination, and ripple marked bedding surfaces displaying desiccated mud drapes within their troughs. Palaeocurrent data suggest that the coastline lay to the north. The unconformably overlying Mozaan Group is entirely composed of siliciclastic sediments of tidal origin and contains sedimentary structures similar to those noted in the Insuzi.

The stromatolites occur within Insuzi carbonates and resemble those recorded from the northern Slave Province (2.5 Gyr) of Canada<sup>3</sup> as they are generally of low relief, rarely rising more than a few cm above the contemporary substrate. They form undulatory surfaces which are accentuated by the partial silicification of the host dolomite. Silicification was selective and not wholesale, so that instead of destroying the finer fabric of the rock this has been enhanced. The stromatolites commonly form low domes

### 3-Gyr-old stromatolites from South Africa

WE report here a new record of early Precambrian stromatolites from the 3.0-Gyr Pongola Supergroup of South Africa (Fig. 1), which is exposed in parts of the south-eastern Transvaal, Swaziland, northern Natal and Zululand. These rocks occur in an almost complete section found in the Wit-Mfolozi River valley, about 200 km north of Durban. In that part of Natal the volcanic-sedimentary Pongola Supergroup attains a maximum thickness of about 2,500 m. It is subdivided into two distinct north-east-dipping



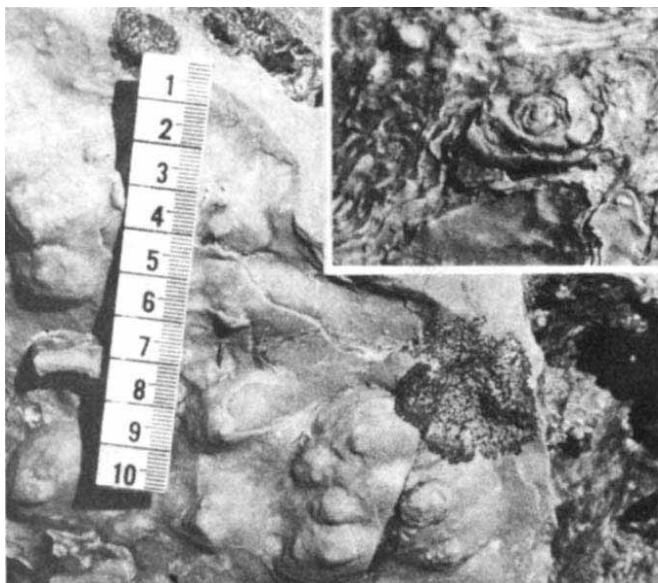
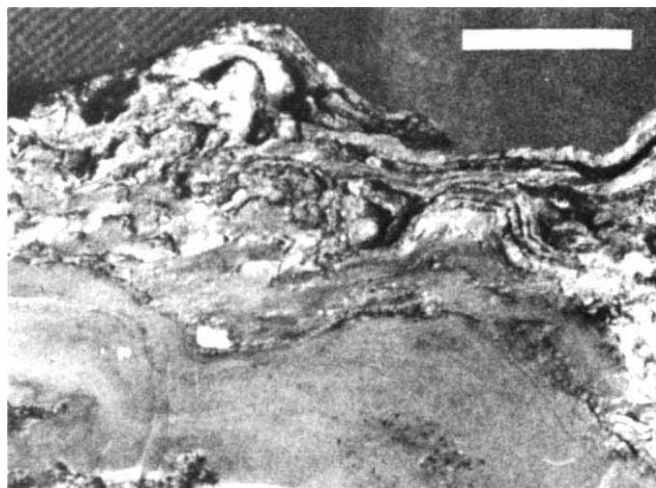


Fig. 2 Low relief domical stromatolites exposed on a bedding surface. Inset at same scale, exfoliated dome showing laminated structure.

(Figs 2, 3) and may be classified according to their geometry<sup>4</sup>. LLH-S, LLH-C and SH types have been recognised. The structures are thought to be of biogenic origin for a number of reasons. First, the preponderance of convex-upwards structures are strongly reminiscent of the growth habits of Recent algal communities. Second, the ubiquitous fine mm scale laminations in the rock are considered to be diagnostic of former algal growth<sup>5</sup>, a conclusion reinforced by microscopic examination which reveals occasional fine sandy layers trapped between successive laminae (Fig. 4). A direct analogue of this feature may be found in the sediment trapping and binding habits of Recent blue-green algal mats<sup>6</sup>. Finally, some of the samples also display fenestral textures, with coarse sparry dolomite filling former voids in the rock. In Recent examples of algal sediments fenestrae are interpreted as structures formed by the interaction of algal mat and algal bound sediment. Desiccation, oxidation and lithification are also important factors leading to the formation of fenestral textures<sup>7</sup>.

Characteristic sedimentary structures in the dolomites provide further evidence of shallow-water sedimentation. In the quartz arenites large dolomite clasts are found (about 30 cm) incorporated in the sandy sediment. They seem to

Fig. 3 Weathered surface showing a section through stromatolite structures. Note the differential erosion of the silica and carbonate layers, and the very fine lamination in the more massive silica at the bottom of the photograph. Scale bar 1 cm.



have been reworked from adjacent carbonate mud flats by currents of considerable velocity. In addition, intraclastic dolomites suggest that there was significant penecontemporaneous reworking of the carbonates and, as these rocks contain herringbone cross lamination, tidal action was the most likely cause.

Insuzi Group stromatolites are of particular interest considering the scarcity of biogenic structures of this age in the geological record. Five comparable occurrences are documented, two in Canada<sup>8,9</sup> and three in Rhodesia<sup>9,10</sup>. The age of the Insuzi Group is not precisely defined. The overlying Mozaan Group rests with a sedimentary contact on the 3.06-Gyr (Rb/Sr) Lochiel granite of the southeastern Transvaal<sup>11</sup>, and is intruded by the Usushwana Complex dated at 2.87 Gyr (ref. 12). Zircons from the Insuzi lavas at Nkandhla gave an age of  $3.09 \pm 0.09$  Gyr (ref. 13) and a whole rock Rb/Sr age of  $3.15 \pm 0.15$  Gyr is reported from Insuzi felsites<sup>13</sup> in the Mozaan valley of Swaziland. From the foregoing evidence there seems little doubt that the Insuzi Group is approximately 3.0-Gyr-old. Comparing this date with the ages generally quoted for the other five localities we find that the age of the stromatolites at Steeprock, Ontario, is in dispute, while those of the northern Slave Province<sup>3</sup> (2.5 Gyr) post-date the Pongola. Work on the Rhodesian greenstone belt<sup>10,14,15</sup> suggests that the Bulawayan and Belingwe stromatolites are about 2.6–2.7-Gyr-old. Assuming that this age is correct, and that it does not represent the resetting of the radiometric clocks by the most recent metamorphic event, the Insuzi stromatolites are the oldest so far documented.

Considering the criteria of emergence and shallow water sedimentation found in the quartz arenites in the Wit-Mfolozi section and the evidence for marine deposition provided by the stromatolites, there is little doubt that the

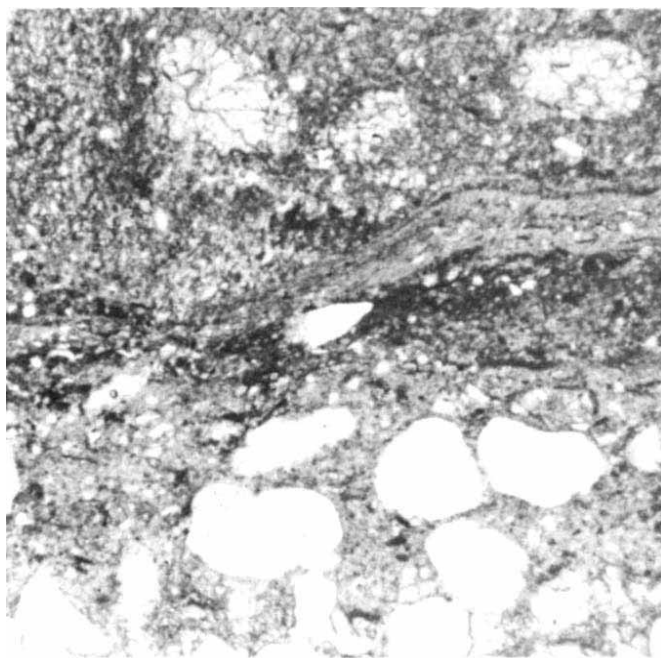


Fig. 4 Fine sandy layer trapped within laminated stromatolite. Note sparry dolomite filling former voids in the rock. Magnification  $\times 70$ .

Insuzi Group was deposited in a tidally influenced environment. Von Brunn and Hobday<sup>16</sup>, applying Klein's<sup>17</sup> model to other Pongola exposures in this region, concluded that the palaeotidal range lies between 12 and 25 m. In the area under discussion comparable palaeotidal amplitudes are suggested by similar sedimentary sequences. Such large amplitudes may have relevance to hypotheses concerning the capture of the Moon by the Earth. The relationship



between the stromatolite-bearing carbonates and evidence of extensive volcanicity is also of note. In all the other early Precambrian occurrences, stromatolites are associated with lavas, and we speculate that the exhalative activities of lava extrusions provided optimum conditions for stromatolite growth. The Earth's atmosphere at the time was probably anaerobic<sup>18</sup>, and some Recent algal species can thrive in such an environment. If ancient stromatolites produced oxygen by photosynthesis, this could have been immediately removed from the environment as free oxygen, and transported to one of the oxygen sinks then operative. The thin banded ironstones in the overlying Mozaan might owe their presence to the local production of biogenically derived oxygen during those times.

Cloud<sup>19</sup> adapted many of his former ideas concerning the major features of crustal evolution to account for the anomalous presence of banded ironstones and stromatolites in the Pongola. He suggested that the events taking place in southern Africa in the early Precambrian were out of phase with those of the rest of the planet in that they anticipated many later developments in other areas.

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## Pigmentation of the ladybird beetle *Coccinella septempunctata* by carotenoids not of plant origin

MANY insects contain carotenoid pigments<sup>1</sup>, and some notable examples are found in the Coleoptera, where carotenoids are responsible for the yellow colour of the yellow and black striped wing cases of the Colorado beetle, *Leptinotarsa decemlineata*, and the orange-red colour of the ladybird beetles, *Coccinella* spp. There has, however, been no report of any insect species being able to synthesise carotenoids *de novo*, and it is generally accepted that in insects, as in other animals, any carotenoids present are of dietary origin<sup>2</sup>. In July 1976 the Liverpool area, like many regions of the UK, experienced an invasion by enormous numbers of ladybirds, almost entirely of the seven-spot variety, *Coccinella septempunctata*. With such large numbers of insects available we were able to use modern analytical techniques to identify the carotenoids of these beetles. We report here that the carotenoids identified were

mostly hydrocarbons, and the carotenoid pattern indicated that these pigments in the ladybird are likely to be of microbial rather than plant origin, thus suggesting the involvement of symbiotic microorganisms.

Preliminary thin-layer chromatographic analysis showed that the carotenoid composition of isolated red wing cases (elytra) was the same as that of the entire insect, so whole insects were used in the large-scale analyses. Carotenoid hydrocarbons formed the bulk of the pigment, but there were also small amounts of many xanthophylls, of which only lutein ( $\beta$ ,  $\epsilon$ -carotene-3,3'-diol) was identified with certainty. The carotenes were characterised by chromatographic properties, light absorption and mass spectra, and whenever possible by comparison with authentic samples. Eighteen compounds were identified, including  $\beta$ -carotene ( $\beta$ ,  $\beta$ -carotene), previously reported by Lederer<sup>3</sup> and by Valadon and Mummery<sup>4</sup>. One of the main carotenes present was torulene (3',4'-didehydro  $\beta$ ,  $\psi$ -carotene), and smaller amounts of  $\gamma$ -carotene ( $\beta$ ,  $\psi$ -carotene),  $\beta$ -zeacarotene (7',8'-dihydro- $\beta$ ,  $\psi$ -carotene), 'cyclic  $\zeta$ -carotene' (7',8',11',12'-tetrahydro- $\beta$ ,  $\psi$ -carotene), lycopene ( $\psi$ ,  $\psi$ -carotene) and 3,4-didehydrolycopene (3,4-didehydro- $\psi$ ,  $\psi$ -carotene) were present. A second series of compounds present possessed a methylenecyclohexane ring system ( $\gamma$ -ring) (ref. 5), a very unusual structural feature.  $\beta$ ,  $\gamma$ -Carotene,  $\gamma$ ,  $\gamma$ -carotene,  $\gamma$ ,  $\psi$ -carotene, 3',4'-didehydro- $\gamma$ ,  $\psi$ -carotene, 7',8'-dihydro- $\gamma$ ,  $\psi$ -carotene and 7',8',11',12'-tetrahydro- $\gamma$ ,  $\psi$ -carotene were all identified. The natural occurrence of the last three of these carotenoids has not been reported previously. A series of more saturated acyclic compounds that are intermediates in carotenoid biosynthesis<sup>6</sup> was identified—phytoene (7,8,11,12,7',8',11',12'-octahydro- $\psi$ ,  $\psi$ -carotene), phytofluene (7,8,11,12,7',8'-hexahydro- $\psi$ ,  $\psi$ -carotene), neurosporene (7,8-dihydro- $\psi$ ,  $\psi$ -carotene) and ' $\zeta$ -carotene' (a mixture of symmetrical 7,8,7',8'-tetrahydro- $\psi$ ,  $\psi$ -carotene and unsymmetrical 7,8,11,12-tetrahydro- $\psi$ ,  $\psi$ -carotene). The carotenoids present in the largest amounts were torulene and  $\beta$ ,  $\beta$ -,  $\beta$ ,  $\gamma$ -,  $\beta$ ,  $\psi$ - and  $\gamma$ ,  $\psi$ -carotenes.

Phytoene, phytofluene, ' $\zeta$ -carotene', neurosporene, 'cyclic  $\zeta$ -carotene' and  $\beta$ -zeacarotene are possible intermediates (as are lycopene and  $\gamma$ -carotene) in the biosynthesis of  $\beta$ -carotene, and have not been detected before in any insect or other animal. The presence of lycopene in the ladybird has finally been proved, long after evidence for its occurrence was first reported<sup>3</sup>. The  $\gamma$ -ring carotenoids and the carotenes of the torulene series are also unusual and their presence in an animal is surprising.

The question of the origin of these pigments cannot be answered in terms of the accepted view that insect carotenoids are of dietary origin<sup>2</sup>. The food chain involving the ladybird is short and simple. The beetles feed on aphids which in turn feed on the juices of green plant tissues. Within this food chain only the green plants are known to be capable of synthesising carotenoids, and there is no known metabolic process whereby the bicyclic compounds with  $\beta$ - and  $\epsilon$ -rings, produced by the plants, could be transformed into acyclic, monocyclic or  $\gamma$ -ring carotenes. The ladybird carotenes must therefore originate elsewhere.

The  $\gamma$ -ring carotenes,  $\beta$ ,  $\gamma$ -carotene and  $\gamma$ ,  $\gamma$ -carotenes, together with  $\beta$ -carotene have, however, been isolated from a green variant of the aphid, *Macrosiphum lirioidendri*, whereas pink variants of the same species contained  $\beta$ -carotene,  $\gamma$ -carotene, torulene, lycopene and 3,4-didehydrolycopene<sup>7</sup>. We stress that the ladybirds used in our investigation were members of a huge, migratory, starving population, and nothing was known of their previous food intake. Although it is unlikely that the ladybirds or their larvae would have had access to the aphid species *M. lirioidendri*, which seems to be a specific parasite of the tulip tree (*Liriodendron tulipifera*), it is not unreasonable to expect that the  $\gamma$ -ring and torulene series carotenoids may be present in other species of aphid which may have formed part of their diet. Even so, the aphids themselves could not obtain these carotenes from the green plants on which they feed.

We therefore conclude that these carotenes are synthesised

within the aphid and/or the ladybird. The possibility of synthesis *de novo* by the insect cannot be ruled out, although this would be the first instance to be discovered of carotenoid synthesis by any animal. The involvement of symbiotic microorganisms seems more probable, and the nature of the carotenoids present gives indirect support for this theory. Apart from these reports of their occurrence in the insect species,  $\gamma$ -ring carotenoids have been found only in a discomycete fungus *Caloscypha fulgens*<sup>8</sup>; torulene and related compounds are the characteristic pigments of red yeasts (*Rhodotorula* spp.)<sup>9</sup>. Further, the ' $\zeta$ -carotene' isolated from ladybirds was a mixture of the symmetrical and unsymmetrical isomers, a situation encountered commonly in bacteria and fungi but not normally found in higher plants<sup>10</sup>. Weisgraber *et al.*<sup>11</sup> have summarised evidence which they believe suggests strongly that the  $\gamma$ -ring and torulene series carotenes present in the aphid *M. liriiodendri* are of microbial origin, and aphids in general are rich in symbionts (yeasts, bacteria, etc.) some of which are red<sup>12</sup>.

For the ladybird, many of the carotenes present could presumably have been derived from aphids (or their symbiotic microbes) devoured by the beetles, but it seems unlikely that phytoene and the other desaturation and cyclisation intermediates would be absorbed and accumulated by the ladybird if they were obtained only in minute amounts in its food. Their presence suggests a site of synthesis within the ladybird itself. Although no information is available about the microflora of the ladybird, it is reasonable to believe that carotenogenic microorganisms present in its aphid prey could be acquired and cultivated by the beetle. Thus it is likely that the carotenoids responsible for the characteristic red colour of *C. septempunctata* are synthesised within the insect, probably through an association with symbiotic microorganisms. Preliminary analysis indicates that the situation may be similar in other species of ladybird.

We thank Dr Noël Arpin for providing  $\beta$ , $\gamma$ -carotene from *Caloscypha fulgens* for comparison.

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and photoevolution of hydrogen. A major obstacle to combined biochemical and genetic studies of these processes is a lack of knowledge of gene transfer in such bacteria. The only transfer mechanism known is that reported by Marrs for the facultative phototroph *Rhodospirillum rubrum* *capsulata*. In this bacterium gene transfer is mediated by a filterable agent of small size (Gene-Transfer-Agent) estimated to carry between  $10^3$  and  $10^6$  daltons of DNA; the nature of these GTAs is unknown. Although GTAs have been used to construct a preliminary map of the bacteriochlorophyll and carotenoid biosynthetic genes in *R. capsulata* (ref. 2), the small amount of DNA transferred limits their usefulness in genetic analysis. To obtain alternative means of gene transfer we examined isolates of the closely related species *R. sphaeroides* for temperate transducing bacteriophage and conjugative R plasmids.

Several temperate, but evidently non-transducing bacteriophage have been isolated from *R. sphaeroides* (ref. 3) and *R. capsulata* (ref. 4). Many of our wild type isolates carry between one and three prophages and more than 90% are resistant to penicillin because of a diffusible penicillinase (unpublished data). Since the production of diffusible penicillinases by *Staphylococcus aureus*<sup>5</sup>, *Pseudomonas aeruginosa*<sup>6</sup> and various enteric bacteria<sup>7</sup> is encoded by plasmid genes, extrachromosomal DNA isolated from *R. sphaeroides* (refs 8, 9) could constitute extrachromosomal prophages and/or penicillinase plasmids.

Initial experiments with a penicillinase-producing strain of *R. sphaeroides* RS601 (prototrophic, penicillin resistant) showed that a sensitive mutant RS602 (prototrophic, penicillin sensitive), isolated after treatment with the mutagen nitrosoguanidine, was 'cured' of a prophage. Bacteriophage released spontaneously by the wild type RS601 ( $10^3$ – $10^4$  plaque-forming units per ml of supernatant) produced small (1 mm diameter) turbid plaques on the 'cured' derivative RS602.

Although attempts to perform generalised transduction with this bacteriophage (designated R $\phi$ 6P) were unsuccessful, when recipient cells (RS602) were grown in aerobic conditions at 32 °C and at a multiplicity of infection of 1.0, 50% of 'survivors' of bacteriophage infection were penicillin resistant. An analysis of 100 penicillin resistant transductants derived from this experiment showed that each produced a diffusible penicillinase.

The question now arises as to the exact nature of this high frequency transduction; at least two explanations seem possible. Either the transduction is mediated by a defective specialised transducing bacteriophage analogous to P11de in *Staphylococcus aureus*<sup>10</sup>, or the wild type bacteriophage

Table 1 The relationship between penicillin resistance and lysogenisation by R $\phi$ 6P in *R. sphaeroides*

| Possible phenotypes                  | Number of colonies |
|--------------------------------------|--------------------|
| Pen <sup>+</sup> $\phi$ <sup>−</sup> | 1127               |
| Pen <sup>+</sup> $\phi$ <sup>+</sup> | 0                  |
| Pen <sup>−</sup> $\phi$ <sup>−</sup> | 0                  |
| Pen <sup>−</sup> $\phi$ <sup>+</sup> | 123                |

An exponential culture (about  $5 \times 10^8$  colony forming units per ml) of RS602 (penicillin sensitive, R $\phi$ 6P sensitive) grown with aeration at 32 °C, was infected with R $\phi$ 6P at a multiplicity of 0.1. The bacteriophage were allowed to adsorb for 30 min at 32 °C without aeration and then excess free bacteriophage were removed with anti-bacteriophage antiserum. The bacteriophage infected cells were diluted and plated on PYEA (0.3% peptone, 0.3% yeast extract, 1.5% agar) to give approximately 50 colonies per plate. After 2 d of incubation at 32 °C the colonies were patched on to PYEA plates and later replicated onto PYEA + 1  $\mu$ g ml<sup>−1</sup> penicillin to determine their resistance/sensitivity phenotype. Lysogeny, as determined by release of free bacteriophage, was assayed by overlaying the patch plates with soft PYEA (0.8% agar) seeded with RS602.

## Naturally occurring viral R plasmid with a circular supercoiled genome in the extracellular state

PHOTOSYNTHETIC bacteria have been used extensively in studies on photosynthesis, photochemical nitrogen fixation



carries a gene for penicillinase production. In experimental conditions which limit infection to a single bacteriophage per cell, there was a 100% correlation between the presence of wild type bacteriophage and production of penicillinase (Table 1). Hence the penicillinase gene is probably carried as part of a wild type bacteriophage genome.

In view of the importance of plasmid coded penicillinase activity we explored the possibility that R $\phi$ 6P exists as a plasmid in the lysogenic cell. A simple test used to differentiate plasmid and chromosomal genes is the Arber experiment<sup>11</sup>. Low level ultraviolet irradiation of transducing bacteriophage lysates stimulates transduction of chromosomal genes and depresses transduction of plasmid genes. Figure 1 shows a depressed frequency of transduction following irradiation, indicative of an extrachromosomal location for this gene in the transduced (lysogenic) cell. Confirmation on these data is being sought by the isolation and characterisation of plasmid DNAs from lysogenic (R $\phi$ 6P+) and non-lysogenic (R $\phi$ 6P-) derivatives of *R. sphaeroides*.

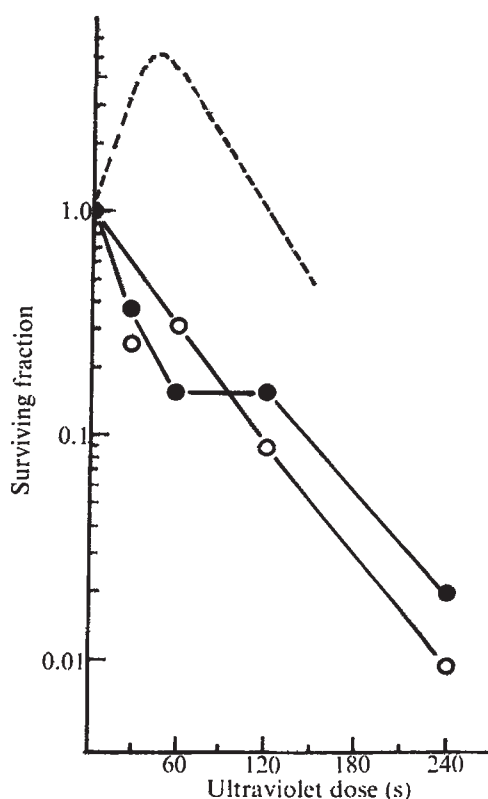


Fig. 1 The effect of ultraviolet-irradiation on the transduction of the penicillinase gene by R $\phi$ 6P. Bacteriophage R $\phi$ 6P was diluted in bacteriophage buffer (ref. 3 to give  $10^8$  plaque-forming units per ml and was ultraviolet irradiated for various lengths of time. Plaque forming titre (○) and transducing titre for the penicillinase gene (●) were determined for each dose of ultraviolet irradiation. The broken curve illustrates the effect of ultraviolet radiation on transduction of chromosomal markers in *Escherichia coli* (ref. 11).

It is unlikely that transforming DNA or gene-transfer agents are involved in this process because transfer is insensitive to DNAase; inactivated by anti-R $\phi$ 6P antisera; and the vector has a molecular weight consistent with the purified bacteriophage particle. An electronmicrograph of the R $\phi$ 6P particle (Fig. 2) shows an isometric head 65 nm in diameter with a long flexuous non-contractile tail 12 nm  $\times$  250 nm, 2–4 tail fibres and no prominent tail plate. DNA molecules obtained from the bacteriophage by phenol extraction<sup>12</sup> and prepared for electron microscopy by the Kleinschmidt technique<sup>13,14</sup>, show a majority of supercoiled molecules suggesting the encapsidated viral genome is circular. Using a variety of means such as DNase, freezing and

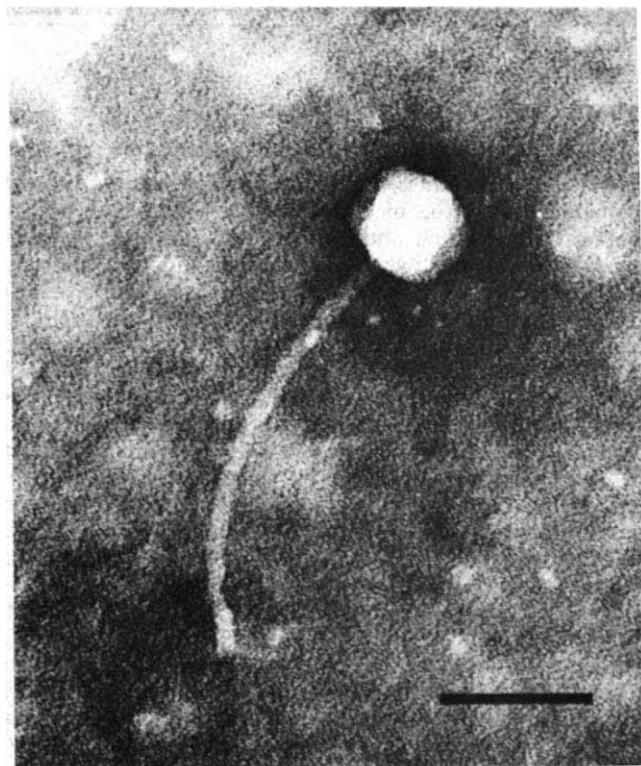


Fig. 2 Bacteriophage R $\phi$ 6P negatively contrasted with ammonium molybdate 1.0% (pH 6.0) using the procedure of Miller, Pemberton and Richards<sup>17</sup>. The bar represents 100 nm.

thawing, alkali and heat treatment, the supercoils could be 'relaxed' to give open circles having a molecular length of  $16.5 \pm 1.0 \mu\text{m}$  which corresponds to a molecular mass of  $33 \pm 2 \times 10^6$  (ref. 15). The only other report of a bacteriophage which possesses a closed circular, supercoiled, extracellular genome is PM2, a bacteriophage whose natural host is a marine pseudomonad<sup>16</sup>.

Richmond<sup>7</sup> states that "purely on statistical grounds bacteriophage conversion and restricted transduction are more likely to play some part in the spread of antibiotic resistance markers than is generalised transduction". This paper records the first instance of naturally occurring phage conversion involving an antibiotic resistance gene. If such virus-R plasmid combinations are widespread in nature then antibiotic therapy directed against drug resistant bacteria may be ineffective in removing the extracellular form of the plasmid from the environment. Finally, R-plasmids possess genes whose functions are still unknown; is it possible that some of these genes encode viral functions?

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## Early presence of ribonucleoprotein antigen on surface of influenza virus-infected cells

Host defence against influenza A virus in experimental animals seems to be mediated mainly by antibody<sup>1,2</sup>. The mechanism by which antibody protects the host, however, is unknown. Antibody may either neutralise viral particles or recognise viral antigens at the surface of infected cells and then act at an early stage of infection, before viral replication is completed. The latter mechanism is theoretically more efficient, but might cause immunopathological destruction of the infected cells with harmful consequences. Viral envelope antigens (haemagglutinin and neuraminidase) have been shown to be present at the surface of influenza virus-infected cells, by haemadsorption<sup>3</sup>, electron microscopy<sup>4</sup> and immunofluorescence<sup>5</sup>. Two main type-specific internal antigens have been described in influenza A virus—the matrix protein (MP) antigen<sup>6</sup> and the ribonucleoprotein (NP) antigen<sup>7</sup>, a non-glycosylated polypeptide with a molecular weight of 58,000 (ref. 8). Immunofluorescence studies on fixed cells have shown that NP antigen is first demonstrable in the cell nucleus and then appears as granular masses within the cytoplasm, which become prominent at the cell borders later in infection, suggesting a close association with the cell membrane<sup>9</sup>. We report here that NP antigen can be detected by immunofluorescence as early as 2 h after the infection on the surface of unfixed mouse cells infected with influenza A virus.

Two methods were used to obtain anti-NP antibodies. The first is described in Table 1. A pool of sera was collected from CBA mice infected intranasally 3 weeks previously with a sublethal dose of an egg-grown A/PR/8 virus. After two cycles of absorption with the homologous concentrated A/PR/8 virus, no detectable antibodies to external components of the virus could be found in this serum, as shown by its negativity in single-radial-diffusion test (SRDT) immunoplates containing intact A/PR/8 virus particles as antigen. The zone area obtained in a SRDT immunoplate containing a disrupted influenza A avian virus (external antigens unrelated to those of A/PR/8 virus), however, was only slightly decreased. Thus, antibodies to internal components of all influenza A viruses were detectable in the serum before and after absorption. At low concentrations of disrupted avian influenza A antigens, the SRDT method reveals exclusively anti-MP antibodies when an anti-human influenza A serum is tested<sup>10</sup>. Comparison of results in SRDT with high and low concentrations of

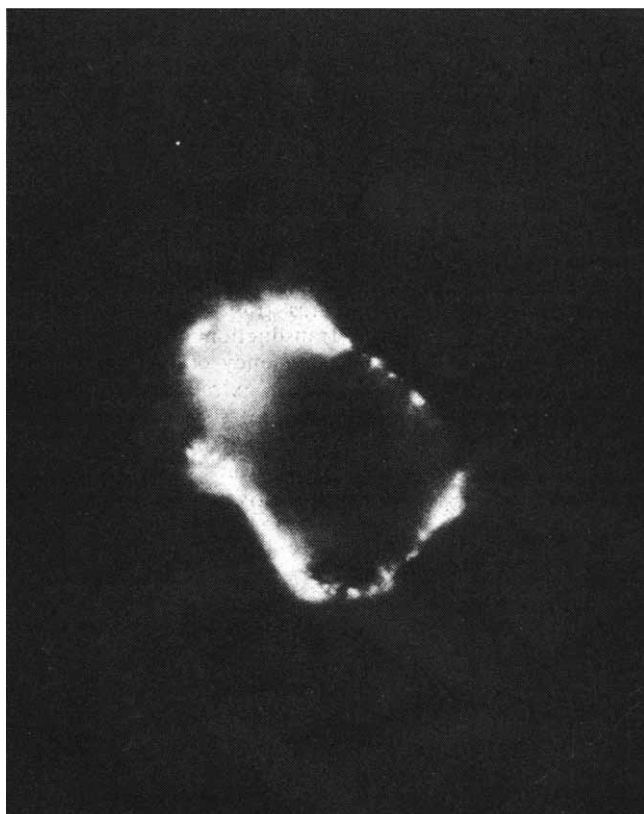


Fig. 1 Ring of immunofluorescence around a D55 mouse cell infected 3 h previously with influenza A/turkey/England/63 and treated with an anti-influenza A NP antigen (mouse anti-A/PR/8 serum absorbed with A/PR/8 particles) and FITC conjugated goat anti-mouse immunoglobulins.

avian influenza A antigens indicated that anti-NP, but not anti-MP antibodies, were detectable in this serum. The second method to obtain anti-NP antiserum from rabbits immunised with NP antigen isolated after electrophoresis of influenza A/Hong-Kong/68 (H3N2) on cellulose acetate paper strips has been described before<sup>11</sup>.

For indirect surface immunofluorescence, unfixed, infected D55 mouse cells adherent on glass coverslips were treated as described in Table 2. Uninfected cells or infected cells incubated with fluorescein isothiocyanate (FITC) conjugated anti-immunoglobulins sera alone were negative. Similarly, control cultures infected with B/Lee virus (an influenza B virus with no cross reactivity with external or internal components of influenza A viruses) were negative.

Table 1 Specificity of anti-influenza antibodies in a mouse convalescent anti-A/PR/8 serum after absorption with A/PR/8 virus

| Antigen in SRDT immunoplate*                                                                       | Specificity of antibodies detectable with immunoplate   | Zone area before absorption (mm <sup>2</sup> ) | Zone area after absorption with A/PR/8 virus† (mm <sup>2</sup> ) |
|----------------------------------------------------------------------------------------------------|---------------------------------------------------------|------------------------------------------------|------------------------------------------------------------------|
| Purified-concentrated A/PR/8 virus (15 mg ml <sup>-1</sup> in agar)                                | Anti-external antigens (haemagglutinin + neuraminidase) | 10.0                                           | 0                                                                |
| Purified-concentrated and SSS detergent disrupted avian N virus (1.5 mg ml <sup>-1</sup> in agar)  | Anti-internal antigens (NP + MP)                        | 10.7                                           | 8.8                                                              |
| Purified-concentrated and SSS detergent disrupted avian N virus (0.15 mg ml <sup>-1</sup> in agar) | Anti-MP                                                 | 0                                              | 0                                                                |

\*A/PR/8 antigens were intact whole particles. The A/chicken/N/Germany/49 (Hav 2 Neq 1) was disrupted by mixing with equal volume of 1% sodium-sarkosyl sulphate (SSS) for 5 min before being mixed with 3 ml of agar.

†Equal volumes of anti-PR8 serum and of a purified-concentrated suspension of A/PR/8 virus (10 mg viral proteins per ml) were incubated for 1 h at 4 °C and centrifuged at 100,000g for 1 h. This procedure was repeated twice. The final supernatant was absorbed twice with packed chicken erythrocytes and heated at 56 °C for 1 h to remove all remaining viral particles. At the end of the process, an haemagglutination test was negative (<1/2).

Table 2 Surface immunofluorescence detected by anti-NP antibodies in mouse cells infected with influenza virus

| Infecting virus | Mouse anti-A/PR/8 serum absorbed by A/PR/8 virus |     |     | Rabbit anti-NP serum |     |     | Haemadsorption |     |     |      |
|-----------------|--------------------------------------------------|-----|-----|----------------------|-----|-----|----------------|-----|-----|------|
|                 | 2 h                                              | 3 h | 4 h | 2 h                  | 3 h | 4 h | 2 h            | 3 h | 4 h | 12 h |
| B/Lee           | 0                                                | 0   | 0   | 0                    | 0   | 0   | 0              | 0   | *   | ***  |
| A/PR/8          | 0                                                | 0   | *   | 0                    | 0   | *   | 0              | 0   | 0   | *    |
| A/Turkey/Eng.   | *                                                | *** | **  | *                    | *** | **  | 0              | 0   | *   | ***  |

Non-confluent monolayers of cells from the D55 mouse permanent cell line adherent on glass coverslips were infected with influenza B/Lee, A/PR/8/34 (HONI) or A/Turkey/England/63 (Hav 1 Nav 2) at about  $100 \times 10^5$  per cell. At various intervals after infection the coverslips were washed thoroughly in cold phosphate-buffered saline (PBS). The cells were not fixed. After 30 min incubation at 4 °C with either inactivated mouse anti-A/PR/8 serum absorbed with A/PR/8 virus (see Table 1) or a rabbit serum anti-purified NP from A/Hong-Kong/68, the cultures were incubated for a further 30 min with (respectively) FITC-conjugated goat anti-mouse immunoglobulins serum or swine serum anti-rabbit immunoglobulins. After 1 h washing in cold PBS, they were mounted on glass slides with 50% glycerol phosphate. Surface immunofluorescence was recorded as absent (0), slight (\*) intense (\*\*) or very intense (\*\*\*). Haemadsorption was performed by adding a suspension of 1% chicken erythrocytes for 30 min at 4 °C followed by washings in PBS.

This was not due to lack of production of influenza B antigens since B/Lee infected cells were able to haemadsorb chicken erythrocytes as early as 4 h after infection, and gave an intense haemadsorption 12 h after infection. A bright, 'patchy' immunofluorescence was found all over the surface of cells infected with either A/PR/8 or A/turkey virus (Fig. 1). This immunofluorescence was detected as early as 2 h after infection in A/turkey infected cells, increased rapidly and was maximal 3 h after infection, when the pattern was a complete ring of fluorescence around the cells. There was no evidence of capping. The fluorescence began to fade 4 h after infection, when the first haemagglutinins were appearing on the surface as shown by the slight positivity of the haemadsorption test at that time. The fluorescence was weaker and appeared later in A/PR/8 than in A/turkey infected cells. The negativity of haemadsorption at 4 h, and the modest positivity of haemadsorption found at 12 h suggest that A/PR/8 virus was poorly infective in D55 cells; this could explain the more discrete immunofluorescence found in these cells. Surface immunofluorescence was also detected with a rabbit anti-NP antiserum in primary kidney cells, Vero cells and MDBK cells infected with influenza A/FPV, A/NWS and A/HK/1/68 virus.

Our results strongly suggest that NP antigen is present on the surface of cells infected *in vitro* with influenza A virus. Thus two anti-NP sera gave a fluorescence on unfixed cells infected with an avian virus with external antigens unrelated to those of the viruses used to raise the antisera. But, two more internal structural polypeptides have been described in influenza A virus, P1 and P2 (ref. 8). The possibility that P1 and P2 may be present as minor contaminants of the NP antigen as separated on cellulose acetate strips cannot be excluded. At present, there is no way to detect selectively anti-P1 or P2 antibodies. The presence of such antibodies in our convalescent mouse anti-influenza serum, however, seems unlikely. Finally, non-structural antigens induced by influenza A virus have been detected by immunofluorescence in nucleoli of infected cells<sup>12</sup>, and two non-structural viral polypeptides have been described<sup>13</sup>. If we fixed our cells with acetone, however, NP antigen was detected in the nuclear and paranuclear area of the cells, but there was no immunofluorescence in the nucleoli. The presence of antigens related to the virus core on the cell surface is not confined to influenza virus, since analogous observations have been made with oncornaviruses<sup>14</sup>. Presence of these antigens early in infection may facilitate assembly with the envelope antigens which enter in the membrane later. Pulse labelling studies with <sup>35</sup>S-methionine have demonstrated the early synthesis of the NP polypeptide in chicken embryo fibroblast cells infected with fowl plague virus and the association of this protein

with membrane fractions of the infected cell<sup>15</sup>. The relevance to host defence mechanisms of our finding that NP antigen is present at an early stage on the surface of influenza virus-infected cells is not known. In theory, this would allow antibody or lymphocytes with anti-NP specificity to destroy infected cells at a stage when the virus has not yet replicated. Since all influenza A viruses share the same NP antigens, such a protective mechanism might cross the subtype barrier. Indeed, our unpublished results, and other reports<sup>16,17</sup> suggest that cross protection can be found between influenza A viruses which do not cross react at the level of their external antigens. The presence of type-specific internal antigens on the surface of infected cells might explain these observations. However, we have so far been unable to protect mice or ferrets by immunisation with purified MP or NP antigens or by passive immunisation with anti-NP or anti-MP antibodies<sup>11,18</sup>. This failure may mean either that the presence of NP-antigen on the surface of infected cells is not relevant to host defence, or that our immunisation technique was not sufficient to elicit an efficient immune response. Indeed a failure to induce protection by passive transfer of anti-NP antibody cannot exclude the possibility that, under physiological conditions, lymphocytes, but not antibody, can recognise NP antigen on the cell surface and protect the host either by direct cytotoxicity or by lymphokine secretion. Further work is needed to determine whether internal viral antigens on the surface of infected cells can be responsible for the paradoxical heterotypic protection observed with influenza A virus.

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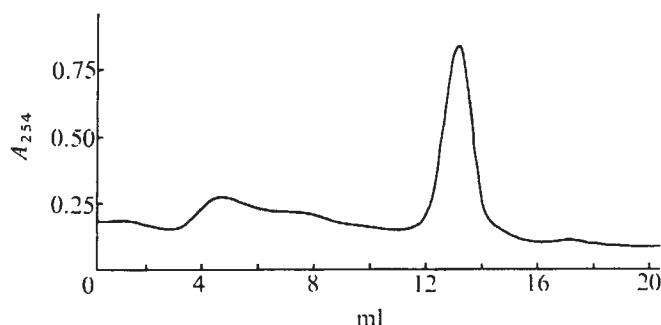
## Infectious DNA from a whitefly-transmitted virus of *Phaseolus vulgaris*

GOLDEN yellow mosaic (bean golden mosaic) is a widespread disease of *Phaseolus vulgaris* in tropical and subtropical America<sup>1</sup>. The disease causes serious yield losses in several countries where beans are an important dietary staple<sup>2,3</sup>. The causal agent, long presumed to be a virus, also infects *P. lunatus* and *Macroptilium lathyroides* in the American tropics<sup>4,5</sup>. The pathogen is transmitted in nature and in the laboratory by the whitefly *Bemisia tabaci* Genn., and is also mechanically transmissible from the sap of infected plants<sup>3,5</sup>. An unusually small (18-nm diameter) nucleoprotein particle has been purified from diseased plants<sup>5,6</sup>, and shown conclusively to be the whitefly-transmitted virus causing the disease<sup>6</sup>. Electron microscopy of purified virus revealed predominately geminate aggregates of the 18-nm particles<sup>5,6</sup>. The virus must be aldehyde-fixed before negative staining because it is disrupted by the heavy metal salts (ammonium molybdate, sodium phosphotungstate, or uranyl acetate) used as negative stains. Thus, it is not known whether the geminate aggregate is the infectious entity or an artefact of fixation<sup>6</sup>. Nonetheless, its unusually small size sets this virus apart from most other plant viruses. I describe here the extraction of infectious DNA from purified golden yellow mosaic virus (GYMV), a result that further separates this virus from other previously described plant viruses.

Highest yields of purified virus were obtained from the leaves of virus-infected plants (*P. vulgaris* 'Top Crop') harvested 12–14 d after inoculation. Purification attempts with diseased tissue collected 19 d after inoculation yielded about 20% as much virus as was obtained at 14 d. The purification procedure (see Fig. 1) effected a complete separation between the virus and phytoferritin, which was present in copious amounts in extracts of GYMV-infected plants. Partially purified virus subjected to rate-zonal centrifugation in linear sucrose density gradients sedimented as a single band (Fig. 1).

The virus collected from density gradients was concentrated by ultracentrifugation and resuspended in 0.1 M sodium phosphate, pH 7.8, for analytical ultracentrifugation. Sedimentation velocity experiments showed a single sedimenting boundary (Fig. 2) with a mean sedimentation coefficient<sup>7</sup> ( $S_{20,w}$ ) of  $69.4 \pm 1.15$ . Attempts to determine the buoyant density of the intact virus in caesium chloride failed because the virus was disrupted by the salt.

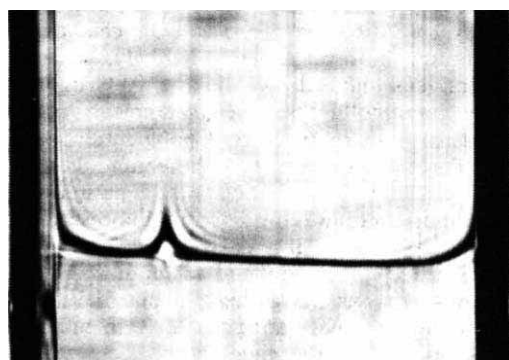
Nucleic acid from GYMV was prepared by dissociating virus with 20 mM Tris-HCl, 5 mM sodium EDTA, pH 9.0, containing 2% (w/v) sodium dodecyl sulphate at 20° for 30 min (ref. 8). Dodecyl sulphate was precipitated by adjusting the mixture to 0.04 M KCl and storing it overnight at 4°. Nucleic acid was recovered from the supernatant liquid after low-speed centrifugation and dialysed overnight against 15 mM sodium citrate, 0.15 M sodium chloride (SSC), pH 7.0. Infectivity was tested by adding a small amount of carborundum to the nucleic acid in SSC and rubbing the mixture on expanding primary leaves of Top Crop beans with a sterilised ground glass spatula. Five days after inoculation typical symptoms of golden yellow mosaic appeared. Plants so inoculated were frozen and homogenised as before, and virus particles with the properties of GYMV (ref. 5) were obtained.



**Fig. 1** Rate-zonal sedimentation profile of purified GYMV in linear 10–40% sucrose gradients. Leaves from infected plants harvested 12–14 d after inoculation were frozen on dry ice, crushed to a coarse powder and homogenised at 4°C in 0.1 M sodium phosphate, 10 mM EDTA, 1 mM cysteine, pH 7.8 (3 ml buffer per g tissue). The homogenate was strained, clarified by centrifugation, adjusted to 0.2 M sodium chloride, and 4 g polyethylene glycol (Carbowax 6000, Union Carbide) was added per 100 ml clarified extract. The polyethylene glycol precipitate was collected by centrifugation after stirring for 2 h at 4°C, and the virus dissolved from the precipitate in a small volume of 0.1 M sodium phosphate, pH 7.8. The virus was further purified by two cycles of differential centrifugation (12,000 r.p.m., 20 min, Sorvall SS-34 rotor followed by 27,000 r.p.m., 3.5 h, Beckman type 30 rotor) and then by rate-zonal centrifugation on linear sucrose density gradients (25,000 r.p.m., 4 h, Beckman SW 27.1 rotor). The sedimentation profile shown was obtained by scanning the sucrose density gradient from the top with an ISCO density gradient fractionator coupled to a Model UA-2 ultraviolet analyser.

The nucleic acid of GYMV sedimented in rate-zonal sucrose density gradients and the analytical ultracentrifuge as a single component (approximately 16S) but did not enter 2.5% polyacrylamide gels. Diphenylamine<sup>9</sup> and orcinol<sup>10</sup> tests were both positive, indicating that the nucleic acid of GYMV might be DNA. This interpretation was confirmed by nuclease sensitivity tests. Nucleic acid treated with bovine pancreatic DNase I (Worthington) was hydrolysed to oligonucleotides, whereas the nucleic acid was unaffected by treatment with RNase A (Sigma) (Fig. 3). The nucleic acid was unaffected by incubation for 4 h at 37°C with 0.3 N sodium hydroxide, a treatment that hydrolyses RNA.

Only four other plant pathogenic viruses have been reported to contain DNA, and GYMV differs from each of these in several important respects. Cauliflower mosaic<sup>11</sup>, dahlia mosaic<sup>12</sup> and carnation etch ring viruses<sup>13</sup> are aphid transmitted and have icosahedral particles of approximately 45 nm diameter. Cauliflower mosaic virus contains double-stranded DNA with a small amount of RNA covalently attached<sup>14</sup>. Potato leaf roll virus, which recent evidence suggests may



**Fig. 2** Analytical ultracentrifuge Schlieren pattern of GYMV. Virus was adjusted to 0.5 absorption units (260 nm) in 0.1 M sodium phosphate, pH 7.8, and centrifuged in an An-D rotor at 35,660 r.p.m. Sedimentation is from the left. Photograph was taken 8 min after rotor reached full speed.

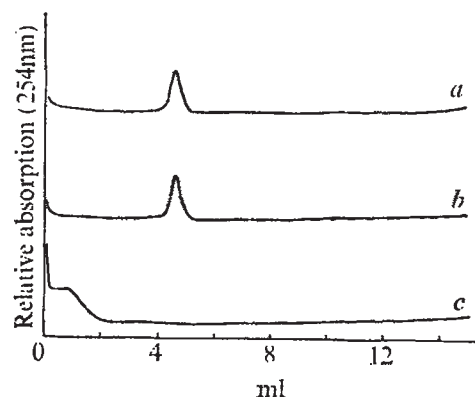


Fig. 3 Rate-zonal sedimentation profiles of GYMV nucleic acid in 10–40% linear sucrose density gradients following various treatments. Nucleic acid prepared in SSC, pH 7.0, as described in the text was adjusted to pH 5.0 and 5 mM  $Mg^{2+}$  by addition of 0.1 M sodium acetate, 50 mM magnesium sulphate, pH 5.0. Nucleic acid concentrations were adjusted to  $10 \mu g \text{ ml}^{-1}$ . To 0.5 ml portions were added 1  $\mu l$  of *a*, water, *b*, RNase A (2.0 mg enzyme per ml), or *c*, DNase I (0.6 mg enzyme per ml water). Mixtures were incubated 4 h at 37 °C and then layered on sucrose gradients in  $9/16 \times 4$  inch cellulose nitrate tubes and ultracentrifuged for 16 h at 25,000 r.p.m. in a Beckman SW 27.1 rotor. Profiles were obtained as in Fig. 1.

contain double-stranded DNA<sup>15</sup>, is also aphid transmitted, and has particles 24 nm in diameter.

GYMV, the only whitefly-transmitted virus so far purified in sufficient quantity for characterisation, is superficially similar to several other viruses or virus-like particles of plants or fungi<sup>16–18</sup>. But there are as yet no reports on the nature of the genome of any of these particles. In size and nucleic acid type, GYMV most closely resembles the parvoviruses, small icosahedral viruses of animals that contain a single-stranded DNA genome<sup>19–21</sup>. Preliminary studies (unpublished) suggest that the DNA of GYMV may also be single-stranded. Thus GYMV and perhaps other whitefly-transmitted viruses may represent an entirely new class of plant pathogenic viruses.

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## Biochemical characterisation of a serum thymic factor

THE search for biologically active peptides with hormonal properties has been intense. The thymus has been looked upon as a source of such materials by a number of investigators but so far no generally accepted compounds have emerged<sup>1–5</sup>. We present here a sequence for a nonapeptide which is found in the serum of several animal species, where its presence is thymus-dependent<sup>6–8</sup>.

For some years we have been attempting to isolate and characterise this material, which we call FTS (*facteur thymique serique*) from the serum of various mammalian species<sup>9–11</sup> in particular the pig<sup>9</sup>; the procedure for extraction has been described in detail elsewhere<sup>9</sup>. Briefly, it consists of dialysis and Sephadex column purification, the stages of which are associated with a progressive increase in biological activity. This has been monitored chiefly by quantification of the effect of the extract on a rosette assay. There is a general correlation between results obtained with this method and with more obviously immunological restoration procedures (refs 9–13). The rosette assay is quick, reproducible<sup>14,15</sup> and amenable to a large-scale testing programme in spite of its somewhat esoteric nature.

Defibrinated pig blood (2,000 l) was ultrafiltered through polyacrylonitrile membranes, concentrated on an Amicon UM2 membrane and subjected to gel filtration on Sephadex G-25 (0.2 M phosphate buffer, pH 7.3). The active fractions were chromatographed on carboxymethyl cellulose (CMC) (0.02 M phosphate buffer, pH 6.3) and eluted with a NaCl gradient in a single peak at 0.12 M NaCl (Figs 1 and 2). Active fractions ( $1.4 \times 10^7$ ) were lyophilised and subjected to gel filtration on Sephadex G-25 packed in 5% acetic acid, and then on Sephadex G-10 in distilled water. The assay of biological activity used at each stage of purification<sup>10,16</sup> is based on the capacity of thymic extracts to render spleen rosette-forming cells from adult thymectomised (Tx) mice sensitive to inhibition by anti- $\theta$  serum and azathioprine. The purity of FTS thus isolated was assessed by (1) the presence of a single spot in thin-layer chromatography (TLC) in five different systems; (2) reproducible stoichiometry of amino-acid composition: Asx, Ser<sub>2</sub>, Glx<sub>2</sub>, Lys, Gly<sub>2</sub>, Ala; (3) the absence of detectable amounts of these

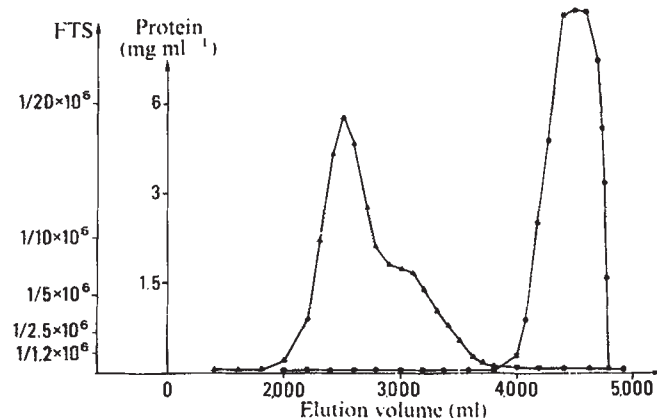


Fig. 1 Elution of pig serum ultrafiltrate on Sephadex G-25 (0.2 M phosphate buffer, pH 7.3). FTS activity was evaluated by the sheep cell rosette  $\theta$  conversion assay. ▲, Proteins; ●, FTS.

amino acids in the control unhydrolysed sample. Sequence studies were performed by Edman's technique as modified by Gray<sup>17</sup>, using dansylation for free amino group determination.

As no  $NH_2$  terminus could be identified in general, the sequence technique was applied to a tryptic digest with the following result: Ser–Glx–Gly–Gly–Ser. The COOH terminus, Asn, was evaluated, after treatment with carboxypeptidase A, by dansylation. In one sample a faint glutamic acid spot was seen



Table 1 Principal biological activities of partially purified natural FTS

|                                                                                                             | Concentration dose       | References |
|-------------------------------------------------------------------------------------------------------------|--------------------------|------------|
| 0 Conversion (Komuro and Boyse assay) <i>in vitro</i>                                                       | 0.1 ng ml <sup>-1</sup>  | 22         |
| Enhancement of the generation of alloantigen reactive cytotoxic T-cells in Tx mice <i>in vivo</i>           | 15 ng per mouse          | 23         |
| Induction of suppressor T-cells in NZB mice assayed using antibody production against polyvinyl pyrrolidone | 15 ng weekly (per mouse) | 13         |
| Enhancement of mitogen responses in nude mice ( <i>in vitro</i> )                                           | 15 ng weekly (per mouse) | 12         |
| in adult Tx rats ( <i>in vivo</i> )                                                                         | 1 ng ml <sup>-1</sup>    | 9          |
| Normalisation of the abnormally high level of autologous erythrocyte binding cells in adult Tx mice         | 75 ng (per rat)          | 24         |
|                                                                                                             | 15 ng (per mouse)        | 11         |

FTS was isolated on the basis of its action on the sheep cell rosette assay<sup>8</sup>.

as the NH<sub>2</sub> terminal residue after dansylation; Edman degradation successively revealed Ala and Lys. The only amino-acid sequence compatible with all data reported above is:



The amino group of residues 1 and 5 is still uncertain. It is likely, because of the difficulty of identifying free amino groups, that most of the NH<sub>2</sub> terminal residues are in the pyroglutamic form at the end of the isolation procedure (whether natively pyroglutamic acid or transformed *in vitro*). Such pyroglutamic acid, however, is apparently biologically active, for the blocked peptide is active in the rosette assay (as indicated by studies of biological material and confirmed by the activity of the synthetic peptide). Residue 5 is probably glutamine rather than glutamic acid in view of the isoelectric point of the peptide (7.3).

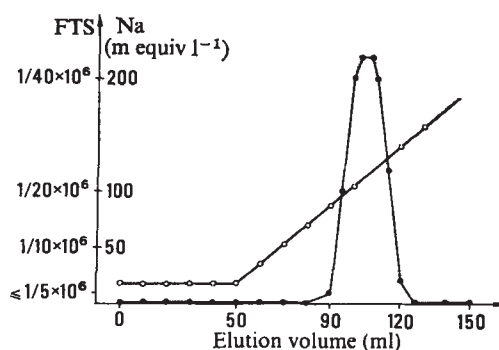


Fig. 2 Elution of Sephadex G-25 eluate after chromatography on CMC (0.02 M phosphate buffer, pH 6.3) and addition of NaCl. ●, FTS; ○, (Na+).

A peptide has been synthesised based on our sequence (with pyroglutamic acid as the NH<sub>2</sub> terminal residue) by the Merck peptide group (West Point, Pennsylvania) using Merrifield's technique of solid phase synthesis<sup>18</sup>. The synthetic peptide has been shown to behave like FTS on TLC (five systems) and had the same elution volume on Sephadex G-25 (0.2 M phosphate buffer, pH 7.3 and 5% acetic acid). Its biological activity was extremely high in the sheep cell rosette assay both *in vitro* and *in vivo*—of the same order of magnitude as natural FTS (Table 2).

In conclusion, we have presented direct evidence for the presence in normal blood of a thymus-dependent nonapeptide of molecular weight about 900, which adds to the list of biologically active peptides which could be hormones. The high activity of the synthetic peptide in the rosette assay of the peptide synthesised on the basis of the amino-acid sequence supports the specificity of its action. Its activity in various systems suggests that the natural peptide is involved in T-cell differentiation. These (Table 1) include (1) 0 conversion *in vitro*<sup>22</sup> and *in vivo*<sup>23</sup>; (2) enhancement of the generation of alloantigen reactive cytotoxic T-cells in Tx mice<sup>13</sup>; (3) induction of suppressor T-cells in NZB mice assayed using antibody production against polyvinyl pyrrolidone<sup>12</sup>; (4) enhancement of mitogen response in nude mice *in vitro*<sup>9</sup> and ATx rats *in vivo*<sup>24</sup>,

and (5) normalisation of the abnormally high level of autologous erythrocyte-binding cells in ATx mice<sup>11</sup>. But the mechanism of such possible FTS involvement in T-cell differentiation is unclear. Most of the biological activities have been demonstrated in adult Tx and NZB mice which suggests that FTS effects the late stages of T-cell maturation. Too little is known to implicate FTS in the first stages of differentiation. It is possible that direct contact between T-cell precursors and thymic

Table 2 Comparison of biological activities of natural and synthetic FTS

|                                                    | Natural FTS                                                               | Synthetic FTS                                                             |
|----------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Sheep cell rosette 0 conversion assay <sup>8</sup> |                                                                           |                                                                           |
| <i>in vitro</i>                                    | 1.5 × 10 <sup>-8</sup> pg ml <sup>-1</sup><br>(1.5 × 10 <sup>-14</sup> M) | 0.7 × 10 <sup>-8</sup> pg ml <sup>-1</sup><br>(0.7 × 10 <sup>-14</sup> M) |
| <i>in vivo</i>                                     | 0.1 ng                                                                    | 0.1 ng                                                                    |
| Autologous erythrocyte-binding cells <sup>11</sup> | 0.2 ng                                                                    | 0.1 ng                                                                    |

epithelium is then necessary whether or not it is mediated by humoral mediators. Alternatively, FTS may not be directly involved in T-cell differentiation in normal conditions and would be classified with previously described pharmacologically immunoactive peptides<sup>19-20</sup> which act in other systems. In any case, FTS might prove useful for the manipulation of T-cell subsets *in vivo*, particularly in clinical conditions showing subtle T-cell deficiency, probably involving the last stages of T-cell maturation, as is well documented in the spontaneously autoimmune NZB mice<sup>21</sup>.

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## Lymphocytes suppressing both immunoglobulin production and erythroid differentiation in hypogammaglobulinaemia

CELLS blocking immunoglobulin (Ig) synthesis *in vitro* have been found among gradient-isolated blood mononuclear cells of individuals with variable immunodeficiency<sup>1-4</sup>, immunodeficiency with thymoma<sup>1,3,4</sup>, infantile X-linked immunodeficiency<sup>4</sup>, multiple myeloma<sup>5</sup> and Hodgkin's disease<sup>6</sup>. It has been suggested that the suppressor cells block terminal differentiation of human B lymphocytes<sup>7</sup>. The association of red cell aplasia and immunodeficiency states is well documented<sup>8</sup>. A circulating Ig capable of interfering with red cell formation has been demonstrated in several persons<sup>9,10</sup>. The majority of individuals with these disorders, however, have no circulating inhibitor of erythropoiesis. In the present study, lymphocytes from two persons with immunodeficiency with thymoma, one of whom had repeated episodes of red cell agenesis, were cocultivated in assay systems in which human blood B lymphocytes and human bone marrow erythroid precursors differentiate *in vitro*. The results suggest that the 'suppressor' cells observed in certain immunodeficiency diseases may act on both lymphocytic and haematopoietic target cells.

Two patients (S1 and S2) diagnosed as having immunodeficiency with thymoma were studied. Ig values for S1 were: IgG, 180; IgM, 0; IgA, 15 mg%; for S2: IgG, 305; IgM, 23; IgA, 22 mg%. Both individuals have had spindle cell tumours of the thymus removed and are currently receiving intramuscular gammaglobulin. Patient S1 has had three episodes of red cell agenesis documented by a falling haematocrit, absent blood reticulocytes, and absent bone marrow erythroid precursors. Remission was induced with steroids which have been tapered to 5 mg prednisone daily. Patient S2 is haematologically normal. In a previous report<sup>11</sup>, patient S1, referred to as B.D. lacked most surface Igs from her blood lymphocytes, whereas S2 (M.P.) was in the normal range.

Lymphocytes were isolated from sterile blood of patients S1, S2 and controls by defibrination over glass beads followed by centrifugation on a Ficoll-Hypaque gradient<sup>12</sup>, and cultured on the same day. Phagocytic cells were removed by sedimentation over a plastic Petri dish for 60 min at 37 °C. T lymphocytes were isolated by rosette formation with sheep red cells as described previously<sup>13</sup>.

Measurement of Ig synthesis *in vitro* has been described previously<sup>3,14</sup> (for experimental details, see Table 1). Erythropoiesis *in vitro* was studied by determining the effect of the patient's serum and mononuclear cells on erythropoiesis using the number of erythroid colonies formed by normal human erythroid precursor cells in response to erythropoietin (EP) in the plasma clot system<sup>15</sup> (for details, see Table 2).

Cultured S1 and S2 mononuclear cells produced much less Ig *in vitro* than cells of normals in similar conditions. In single cultivation experiments shown on the top three lines of Table 1, the means for Ig production of 19 experiments on 14 normal controls were compared with combined

Table 1 Effect of blood mononuclear cells on Ig production of PWM-activated human lymphocytes *in vitro*

|                     | Lymphocyte donor† | No. of expts‡ | S§ Ig (k+λ) c.p.m. (mean ± s.e.m.) | P§        |
|---------------------|-------------------|---------------|------------------------------------|-----------|
| Single cultivation* | N                 | 19            | 236 ± 29                           | 337 ± 31  |
|                     | S1                | 7             | 6 ± 2                              | 13 ± 5    |
|                     | S2                | 4             | 10 ± 5                             | 40 ± 24   |
| Co-cultivation*     | S1 + N            | 14            | 23 ± 8                             | 53 ± 10   |
|                     | S1 + N(ADH)       | 2             | 37                                 | 42        |
|                     | S1(T)   + N       | 2             | 43                                 | 10        |
|                     | S2 + N            | 3             | 47 ± 12                            | 129 ± 111 |
|                     | N + N             | 4             | 261                                | 402       |

\* In single cultivation experiments  $4 \times 10^6$  isolated mononuclears were cultured for 7 d at a concentration of  $2 \times 10^6$  cells ml<sup>-1</sup> in RPMI 1640 containing 17% foetal calf serum, 1% PWM (Grand Island Biol. Co., Grand Island, New York); and penicillin, streptomycin, and glutamine as described<sup>3,13</sup>. At the end of the culture period the cells were washed, suspended in leucine-free MEM and permitted to incorporate <sup>3</sup>H-leucine (10 μCi per 10<sup>6</sup> cells) for 4 h. Ig was determined on Triton-X-solubilised cell pellets and spent culture fluid fractions by a two-stage antibody immunoprecipitation procedure<sup>3</sup>. The mixed antisera had specificity for both kappa and lambda light chains. Data expressed as c.p.m. per 10<sup>6</sup> lymphocytes are after subtraction of control tube. Only experiments in which the control was < 35% of the experimental results were considered valid. Cocultivation experiments were carried out with  $4 \times 10^6$  cells of each cocultivant at  $2 \times 10^6$  cells ml<sup>-1</sup>. Preliminary cocultivation experiments showed no difference in Ig production when the cell concentration was varied from 1 to  $4 \times 10^6$  cells ml<sup>-1</sup>.

† N refers to normal control donors of lymphocytes. S1 and S2 are patients having immunodeficiency with thymoma.

‡ In the single cultivation experiments 14 different normals were involved in the 19 experiments. In co-cultivation experiments, S1 + N involved 6 different combinations of co-cultivants: S2 + N involved 3 different combinations and N + N involved 4 combinations.

§ S refers to the secreted Ig found in the culture fluid after radioincorporation while P refers to the cell fraction.

Adherent cells were removed by leaving isolated mononuclear cells in a shallow plastic Petri dish for 60 min at 37 °C, decanting the cells which remain suspended, and using them. Latex ingestion indicated less than 2% of the remaining cells were macrophages.

¶ S1(T) were T lymphocytes isolated by the sheep red cell rosette method.

PWM is pokeweed mitogen.

results of experiments with S1 and S2. Individual experiments, from which the data in Table 1 were derived, generally included one patient and 1-3 controls tested on the same day. In such individual experiments there was never overlap between the patient and controls; S1 or S2 always produced 30% or less Ig than controls.

Cocultivation experiments between S1 and S2 and normals demonstrated that both S1 and S2 blocked Ig production by cultured normal B lymphocytes. If the data are calculated as Ig c.p.m. as a percentage observed/expected (expected = 1/2 c.p.m. of normal + 1/2 c.p.m. of S1 or S2 in single cultivation), S1 is < 20%; S2 is < 40%. When normals were cocultivated together the observed/expected Ig production was close to 100% of the observed/expected.

Results for secreted and cellular Ig fractions in Table 1 were comparable, suggesting that the defect was not a failure to secrete but rather an inability to synthesise Ig. S1 quantitatively had greater suppressor activity than S2 in single cultivation and cocultivation studies.

Table 2 shows data for the plasma clot erythroid colony testing. In the absence of EP (lines 1-4 of Table 2) no erythroid colonies were formed from bone marrow or lymphoid elements. When EP was added, as seen in line five of Table 2, several hundred colonies were observed. Serum from S1 or S2 did not significantly alter the number of erythroid colonies observed (data not shown). Mononuclear cells from normal subjects enhanced the number of erythroid colonies: the differences noted were significant: the figure in line 6 is the mean of 28 experiments. When  $1 \times 10^6$  lymphocytes from the putative suppressor cells, S1 and S2,



**Table 2** Effect of mononuclear cells on erythroid colony formation by normal human bone marrow *in vitro*\*

| Additions to culture Expt     | Number of colonies per $6 \times 10^5$ cells† |
|-------------------------------|-----------------------------------------------|
| 1 None                        | 0                                             |
| 2 Normal BMC                  | 0                                             |
| 3 S1 BMC                      | 0                                             |
| 4 S2 BMC                      | 0                                             |
| 5 EP                          | $328 \pm 27$                                  |
| 6 EP+normal BMC               | $449 \pm 32$                                  |
| 7 EP+S1 BMC                   | $4 \pm 2$                                     |
| 8 EP+S2 BMC                   | $98 \pm 21$                                   |
| 9 EP+S1 BMC+HC( $10M^{-5}$ )‡ | $16 \pm 3$                                    |
| 10 EP+S2 BMC+HC( $10M^{-5}$ ) | $84 \pm 10$                                   |
| 11 EP+S1 (Adh)§ BMC           | —                                             |
| 12 EP+S1(T)¶ BMC              | 10                                            |
| 13 EP+S2 (Adh)§ BMC           | —                                             |

 $P < 0.005$ \*  $1 \times 10^5$  lymphocytes were used.† Each value represents mean  $\pm$  1 s.e. of 3 separate studies each with 8 individual determinations.

‡ Hydrocortisone.

§ Adherent cells were removed by leaving isolated mononuclear cells in a shallow plastic Petri dish for 60 min at 37 °C, decanting the cells which remained suspended and using them.

¶ T lymphocytes isolated by sheep red cell rosette method<sup>13</sup>.

BMC, Blood mononuclear cells.

Adh, Adherent.

Bone marrow cells obtained from haematologically normal individuals by aspiration from the posterior superior iliac crest were cultured in the presence or absence of 2 IU EP as follows. Each marrow ( $6 \times 10^5$  cells) was cultured with media containing either normal human or patient serum (0.05, 9.1 and 0.2 ml per culture), normal human lymphocytes or patient's lymphocytes (0.5, 1, 2, 4 and  $6 \times 10^5$  lymphocytes per  $6 \times 10^5$  marrow cells). The cultures were carried out for 5–7 d at 37 °C in a humidified atmosphere of 5%  $CO_2$  in air. Only colonies composed of 8 or more erythroid cells were considered in the tabulation of the results.

were added, there was significant decrease in the number of colonies. The degree of suppression is greater for S1 than for S2. Hydrocortisone (lines 9 and 10, Table 2) did not affect the suppression phenomenon.

When adherent cells were removed from S1 and S2 mononuclear cell populations (Tables 1 and 2) there was no loss of suppression in either the Ig production or plasma clot system. Isolated T cells could suppress cocultivated cells in both systems, suggesting that the suppressor cell(s) was a T lymphocyte.

The data demonstrate that lymphoid cells which interfere with the maturation of both human erythroid precursors and of B lymphocytes in culture are present in the blood of two persons with immunodeficiency with thymoma. By contrast, cells from healthy controls stimulate the production of erythrocytes in the plasma clot system and leave Ig production essentially unaffected in the PWM-activated Ig production assay.

T lymphocytes and adherent cells, which suppress Ig production *in vitro*, are present in a number of immunodeficiency diseases<sup>1–7</sup>. Caution must be exercised in interpreting these cells as aetiological in view of recent data which show that suppressor T lymphocytes are part of the normal immunoregulatory elements in the immune response<sup>10</sup>; suppressor cells are induced by lectins and other agents<sup>17</sup>; and suppressor cells are found in the genetically determined disorder, infantile X-linked immunodeficiency, in which an inherited defect determining suppressor cells would be less likely than the presence of such cells as an epiphenomenon. In the present study, the observations that S1 possessed suppressor cells for erythroid cell development while in remission from her anaemia, and S2 had similar cells although haematologically normal, make it unlikely that these cells were the sole cause of the anaemia in S1.

Recent studies have focused on the role of 'suppressor' mononuclear cells in haematological disorders—particularly

disorders in which there is arrest of bone marrow cells. Suppressor cells of erythropoiesis have been found in cases of Diamond-Blackfan syndrome using the plasma clot assay<sup>14</sup>. There are recent observations implicating blood mononuclear cells in selected cases of aplastic anaemia<sup>18,20</sup>. The present paper, however, is the first in which the same patient has been shown to have suppressor cells for both lymphoid and erythropoietic cells.

The lack of change in suppression after removal of adherent cells and its production by T cells is consistent with the suppressor cells being T lymphocytes in both *in vitro* assays. It is not known whether separate cell subpopulations are involved. Dissociation of suppression of Ig production from suppression of erythroid cell maturation has, however, been noted in cases of Blackfan-Diamond syndrome<sup>18</sup>, and in primary immunodeficiency.

The difficulties in evaluating the relationship between 'suppressor' cells and disease states is complicated by the different types of cells which may possess suppressor activity, and the differences between *in vitro* and *in vivo* conditions. Nevertheless, the present data raise the possibility that suppressor lymphocytes play a role in the pathophysiology of diseases in which both lymphocytic and hematopoietic abnormalities coexist.

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## Reduced rates of proteolysis in transformed cells

GROWTH of cells implies a net accumulation of intracellular proteins with the rate of protein synthesis exceeding the rate of protein degradation. It follows that the seemingly uncontrolled growth of transformed cells could be produced by a reduction in intracellular proteolysis without any concomitant increase in protein synthesis. In order to examine this hypothesis we have determined the rates of protein degradation in two pairs of (non-isogenic) cell types—non-growing rat liver hepatocytes

and the chemically transformed Reuber H35 hepatoma, and mouse BALB/c 3T3 fibroblasts and 3T3 fibroblasts transformed with Simian Virus 40 (SV40). We have measured proteolysis over a wide range of insulin concentrations with cells incubated in either an enriched medium or a basal salts medium (step-down conditions). Insulin was chosen because it inhibits intracellular proteolysis in several cell types<sup>1-6</sup> and may have a major role in the normal regulation of proteolysis *in vivo*. We report here that transformed cells have a lower basal rate of proteolysis together with an increased sensitivity towards inhibition by insulin, two factors which will contribute to the rapid growth of such cells.

Cell proteins were labelled by a 16-h incubation with <sup>3</sup>H-leucine and protein degradation was followed after an intervening 3-h chase (Fig. 1). This chase period is desirable for the effective removal of unincorporated <sup>3</sup>H-leucine and essential to allow for the insulin-insensitive degradation of short-lived proteins<sup>4,7</sup>. Leucine (2 mM) was added during both the chase and degradation periods to reduce the specific activity of <sup>3</sup>H-leucine released from labelled proteins and thus suppress any reincorporation of isotope which would cause an apparent reduction in proteolysis.

We found substantial differences between hepatocytes and hepatoma cells in the overall rate and regulation of intracellular proteolysis (Fig. 1). Breakdown of 14% of the labelled hepatocyte proteins occurred during a 4-h incubation in salts medium. This rate is 50% greater than found for Reuber hepatoma cells labelled and incubated in identical conditions. Furthermore, the lower rate of protein degradation in hepatoma cells also occurred when the experiment was performed in Eagle's Minimal Essential Medium (MEM)<sup>8</sup>. Insulin addition at the beginning of the degradation period inhibited intracellular protein degradation in both cell types, but was effective at much lower concentrations in the hepatoma. Indeed the insulin concentration for half maximum inhibition in Reuber

hepatoma cells was approximately  $10^{-12}$  M, over two orders of magnitude less than the concentration required for comparable inhibition of proteolysis in hepatocytes (Table 1). Moreover, across the whole range of insulin concentrations tested, and even at concentrations producing maximum inhibition of proteolysis, rates of protein degradation in the hepatoma cells were still half those found for hepatocytes.

The lower proteolytic rate and higher sensitivity of protein degradation to insulin in hepatoma cells may reflect a difference between growing and non-growing cells rather than a difference between transformed and normal cells. The omission

Table 1 Insulin concentrations for half maximum inhibition of protein degradation

| Cell type                         | Medium                |                       |
|-----------------------------------|-----------------------|-----------------------|
|                                   | Salts                 | MEM                   |
| Rat hepatocytes                   | $7 \times 10^{-10}$ M | $1 \times 10^{-9}$ M  |
| Reuber H35 rat hepatoma           | $3 \times 10^{-12}$ M | $2 \times 10^{-12}$ M |
| 3T3 (BALB/c3T3) mouse fibroblasts | $3 \times 10^{-9}$ M  | $1 \times 10^{-9}$ M  |
| SV40 transformed 3T3              | $2 \times 10^{-10}$ M | $6 \times 10^{-11}$ M |

Values were calculated from the data in Figs 1 and 2.

of serum during the 3-h chase and 4-h degradation periods, however, would restrict growth of the hepatoma line, especially when incubated in a simple salts medium without amino acids. It seems more likely therefore that the differences in proteolytic rate between the two cell types reflect a changed genotype independent of cell growth rates.

This conclusion is supported by data obtained with mouse 3T3 and SV40-3T3 fibroblasts which differ by the insertion of viral genome<sup>12,13</sup>. Proteolysis measurements on these cell types show qualitatively similar results (Fig. 2) to those described for hepatoma cells and hepatocytes. Thus control rates of protein degradation in nutritional step-down

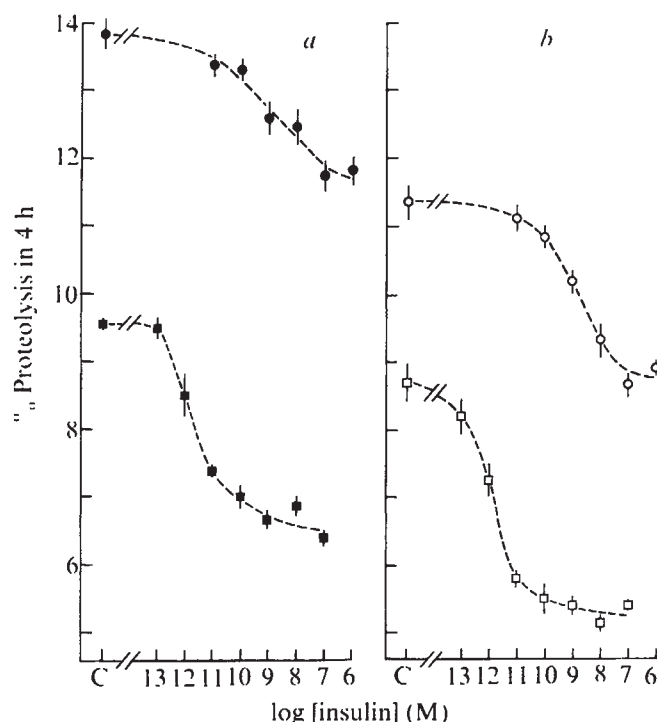
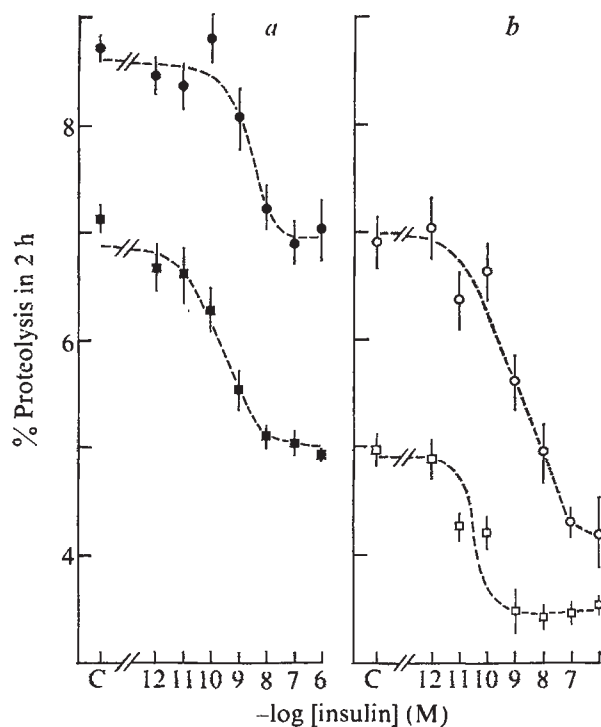


Fig. 1 Proteolysis in hepatocytes and Reuber H35 hepatoma cells. Livers from fed, 150-g rats were perfused with medium containing collagenase to disperse the tissue<sup>8</sup>. Cells were isolated by shaking the dispersed tissue in perfusion medium at 37 °C for 15 min, and filtration through nylon gauze. The cells were pelleted by centrifuging at 50g for 1 min, suspended in calcium-free Eagle's MEM<sup>8</sup> containing 25 mg gentamycin per 100 ml, and transferred to sterile centrifuge tubes. Aseptic techniques were then adopted for subsequent preparation and incubation steps with the isolated cells. The cell suspension was centrifuged as before, the supernatant discarded, and the cell pellet washed once in the same buffer. A final suspension of approximately  $10^6$  parenchymal cells was transferred to 250 ml of MEM containing 20 mM N-Tris [hydroxymethyl]methyl-2-aminoethane sulphonic acid (TES) pH 7.5, 25 mg gentamycin, 25 mg streptomycin sulphate, 15 mg penicillin G, 2.5 mg fungizone and enriched with amino acids and vitamins at twice the recommended amount<sup>9</sup>, together with 10% foetal calf serum (enriched MEM). Aliquots of 5 ml of this diluted cell suspension were dispensed into 60-mm plastic Petri dishes and transferred to a humidified incubator at 36.5 °C and gassed with 5% CO<sub>2</sub> in air. Almost all the hepatocytes attached to the dishes and after 24 h the cells showed a marked morphological change from birefringent spherical cells to translucent flattened cells with clearly visible intercellular abutments. At this time the medium was changed to one with similar constituents but containing <sup>3</sup>H-leucine 1  $\mu$ Ci ml<sup>-1</sup> (specific activity 50 Ci mmol<sup>-1</sup>) and with non-radioactive leucine omitted. The cells were incubated in this medium for a further 16 h to label intracellular proteins. The medium was then changed to MEM containing 20 mM TES and 2 mM leucine but without serum (chase MEM). After a further 3-h incubation the plated cells were washed twice with Earle's salts and incubated either in a, Earle's salts with 20 mM TES and 2 mM leucine added (chase salts) or in b, chase

MEM. Insulin (Actrapid, Novo) was added at the concentrations indicated. After 4 h the cells were collected in medium by scraping with a rubber policeman after addition of Triton X100 to a final concentration of 0.5%, then thoroughly mixed on a vortex mixer and protein was precipitated from 250- $\mu$ l portions by the addition of trichloroacetic acid (TCA) to a final concentration of 20%. The TCA mixtures were kept for 1 h at 0 °C, centrifuged for 10 min at 2000g and the radioactivity in both TCA-soluble and TCA-insoluble fractions measured using NCS Solubilizer (Searle Nucleonics) in a toluene scintillation solution<sup>10</sup>. Proteolysis was expressed as the percentage of total radioactivity in the TCA-soluble fraction (amino acids) after subtracting the TCA-soluble counts at zero time. Reuber H35 hepatoma cells<sup>11</sup>, were grown to a similar density to that obtained with hepatocytes. Subsequent procedures were as for hepatocytes. Values shown for hepatocytes (●, ○) and hepatoma cells (□, ■) are means + s.e.m. for data on between 6 and 10 dishes at each insulin concentration and for control incubations (C) in the absence of insulin.





**Fig. 2** Proteolysis in BALB/c 3T3 and SV40-3T3 cells. The cells were grown in enriched MEM. Labelling, chase conditions and washing were as described in Fig. 1, except for the omission of foetal calf serum during the labelling period. Collecting and radioactivity measurements in cell protein, medium protein, cell amino acids and medium amino acids were as described before<sup>5,10</sup>. Proteolysis is expressed as the percentage of total radioactivity in the sum of the two amino acid fractions. Values shown for 3T3 cells (○, ●) and SV40 3T3 cells (□, ■) are means  $\pm$  s.e.m. for data on 6–15 dishes at each insulin concentration and for control incubations (C) in the absence of insulin. *a*, incubation in chase salts; *b*, incubation in chase MEM.

conditions (salts) are 25% higher in 3T3 compared with SV40-3T3 cells. A slightly greater difference is found when the comparison is made between the two cell types incubated in MEM. Furthermore, proteolysis is more sensitive to inhibition by insulin in the transformed fibroblast than in 3T3 cells. Such differences (Table 1) are less than seen between hepatoma and hepatocytes, but there is still a 15-fold difference in the concentration of insulin required to inhibit protein degradation by 50%.

Lower basal rates of protein degradation and higher sensitivity of the process to insulin inhibition will be important contributing factors towards rapid protein accumulation and growth of cancer cells. The lower basal rate of protein degradation in transformed cells might represent a partially inhibited condition. This is unlikely, however, since the extent of insulin inhibition in transformed cells is at least as great as found for normal cells. Rather, the difference in insulin sensitivity might be due to greater receptor affinity on the plasma membrane of transformed cells. Alternatively, a higher rate of insulin degradation in the non-cancer cells might account for the higher concentration of insulin required to inhibit protein degradation. But, this would occur only if the receptor affinity in the non-cancer cells was great enough to account for the half-maximal effects on degradation in the cancer cells. This does not seem to be the case since the apparent  $K_D$  for insulin binding in both hepatocytes<sup>14</sup> and fibroblasts<sup>15</sup> is lower ( $10^{-8}$  M) than needed to satisfy this restriction, and in good agreement with our calculation of the half-maximum effect of insulin on proteolysis in normal cells. Therefore we consider it most likely that transformed cells have a higher receptor affinity for insulin and that this largely determines their lower rates of protein degradation, although the mechanisms involved remain obscure.

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## Thermogenic defect in pre-obese *ob/ob* mice

YOUNG genetically obese (*ob/ob*) mice develop obesity even when pair-fed to their lean littermates<sup>1–3</sup>. This is due to an unusually high metabolic efficiency, which we have suggested results from a reduction in the “maintenance increment” to the basal metabolic rate (BMR)<sup>4</sup>. Factors involved include the thermic effect of food and the thermogenic response to an environmental temperature below thermoneutrality. Part of the evidence supporting this suggestion comes from the observation that mature *ob/ob* mice, and at least two other strains of genetically obese rodent, are unable to maintain their body temperature when exposed to cold<sup>5–7</sup>. At 4 °C both the *ob/ob* mouse and the Zucker (*fa/fa*) rat rapidly die of hypothermia<sup>5,7</sup>. In the mouse, thermogenesis rather than heat conservation has been established as the cause of the thermoregulatory defect<sup>5,7</sup>. We now report that a defective response to cold is apparent in preweanling mice bearing the *ob/ob* genotype about 2 weeks before they can be identified as obese by visual inspection. From this a test based on the response to 4 °C has been developed for the identification of *ob/ob* mice at 17 d old.

Exploratory studies showed that 17 d was the earliest age at which preweanling ‘normal’ mice can maintain their body temperature when placed in a cold room (4 °C) for up to 90 min. This age was therefore taken as the base-line for comparison of the response to 4 °C of lean and ‘pre-obese’ animals. Litters were derived from lean parents heterozygous for the *ob* gene (*ob/+*) since the obese (*ob/ob*) female is infertile and the obese (*ob/ob*) male is too corpulent to mate. Mice were placed at 4 °C for up to 60 min and their rectal temperature was measured periodically. Each litter consisted of two groups: those in the larger group, although showing some change, could maintain their body temperature within the normal range; those in the

smaller group exhibited a marked decrease and had a lower initial temperature. Temperature response curves of mice from nine litters are shown in Fig. 1. Mice unable to maintain a normal temperature were marked for subsequent identification and all members of each litter were returned to their nest. Each animal was then examined at 28–30 d old, the earliest age at which obese individuals can be identified by visual inspection. In all litters the mice that had been marked were obese and those that were unmarked were lean. Clearly, 17-d-old mice with the *ob/ob* genotype are less able than normal mice to respond to the cold.

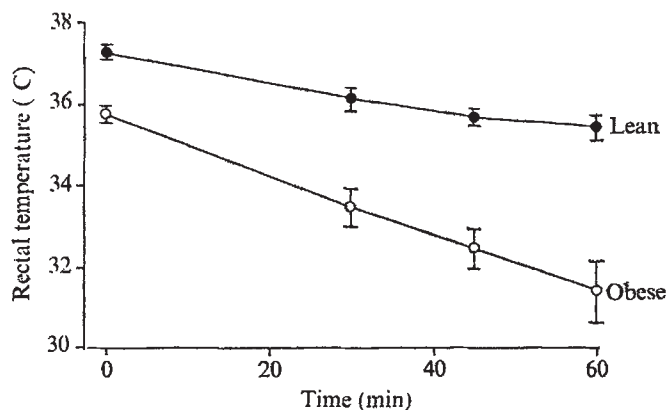


Fig. 1 Rectal temperatures of 17-d-old mice in response to an environmental temperature of 4 °C. Mice were removed from the nest and their initial temperature was taken by inserting a thermocouple (diameter <1 mm) 10 mm into the rectum; the thermocouple was attached to a Comark 3001 digital thermometer. Each mouse was individually caged and immediately transferred to a 4 °C room. Further temperature measurements were taken periodically. The results are expressed as the means  $\pm$  s.e.m. for 39 lean animals (●), and for 16 animals that were subsequently found to carry the *ob/ob* genotype (○). At each time the temperature difference between the two groups was statistically significant,  $P < 0.001$ , on the basis of a Student's *t* test.

More litters were then examined with the aim of using the impaired response to a 4 °C cold stress as a test for the *ob/ob* genotype. Such a test is essential if the metabolic changes preceding the development of obesity are to be elucidated. In total 133 mice from 19 litters were used. Before the experiment 7% were discarded as either abnormally small or in poor condition. The rest were categorised as 'definitely lean' or 'definitely obese' according to how they tolerated the cold. Some of those classified as definitely obese had such a precipitous fall in temperature that they had to be returned to the nest before the full 60-min test was completed. 19.5% of animals were predicted to be obese and 72.4% to be lean; the genotype of all these animals was found to be correctly predicted when they were examined for obesity at 28–30 d (Table 1). A few (8.1%) that could not be categorised readily were termed 'tentatively lean.' These showed a fall in temperature outside the normal range, but much less than for the 'definitely obese' mice. Of the 'tentatively lean' animals, two were found to be obese and eight to be lean. Clearly at 17 d-old, individuals that will be lean or obese can be obtained with certainty from litters derived from heterozygous parents. Genotype was predicted correctly for 98% of animals.

The stressfulness of the method of identification seems not to be a problem, for we have not observed an increased morbidity or mortality in cold-stressed mice, nor have we found long

term effects on their growth rate and reproductive performance. Kaplan and Leveille<sup>8</sup> identified *ob/ob* mice between 18 and 20 d old by their reduced oxygen consumption at 25 °C. The main advantages of our procedure, apart from the marginal improvement in the age at which the genotype can be identified, are its simplicity and the ease with which many animals can be examined in a short time.

To investigate whether the thermoregulatory defect of *ob/ob* mice is detectable earlier than 17 d we examined the response to environmental temperatures less severe than 4 °C in mice aged between 12 and 17 d. Animals younger than 12 d could not be used because even our finest thermocouple probes caused rectal bleeding. With 12 d-old mice and an environmental temperature of between 15 and 20 °C *ob/ob* and lean animals were sharply differentiated. The former showed a marked fall in body temperature within 15 min of exposure to the cold (Fig. 2). The fact that defective thermoregulation occurs in *ob/ob* mice as young as 12 d indicates that a test similar to that for 17-d-old animals could be used for their identification.

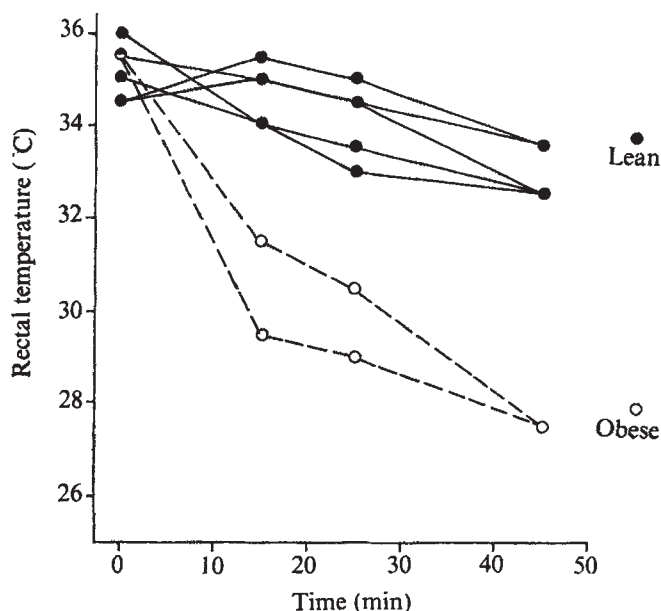


Fig. 2 Rectal temperatures of a litter of 12 d-old mice in response to an environmental temperature of 15 °C. Experimental details are the same as in Fig. 1. The animals fell into two groups, those subsequently found to be *ob/ob* (○) and those found to be lean (●).

The work reported here shows that the defective thermoregulation exhibited by mature *ob/ob* mice is not a secondary consequence of obesity. Because the defect in the adult animals is due to an impairment in the capacity for thermogenesis rather than in heat conservation<sup>6,7</sup>, the same cause presumably applies to the defect in the pre-obese animals. These results therefore support the suggestion that a reduction in the "maintenance increment" of an animal's energy expenditure is a fundamental factor in the increased metabolic efficiency that leads to obesity<sup>4</sup>. In mice non-shivering thermogenesis is a major contributor to energy expenditure when the animals are maintained, as is usual, about 10 °C below their critical temperature. Thus, reduced non-shivering thermogenesis may account for the enhanced metabolic efficiency of *ob/ob* mice.

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Table 1 Prediction of the genotype of lean and *ob/ob* mice aged 17 d by their response to an environmental temperature of 4 °C

| Prediction       | Result |           |          | % Correctly predicted |
|------------------|--------|-----------|----------|-----------------------|
|                  | No.    | No. obese | No. lean |                       |
| Definitely obese | 24     | 24        | 0        | 100                   |
| Definitely lean  | 89     | 0         | 89       | 100                   |
| Tentatively lean | 10     | 2         | 8        | 80                    |



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## Post-weaning food restriction reduces adipose cellularity

KNITTLE and Hirsch<sup>1</sup> reported that varying the caloric intake of newborn rats by manipulating litter size during the 21-d suckling period followed by allowing free access to food after weaning caused the rats from small litters to have more adipocytes in the epididymal depot than rats from large litters. Hirsch and Han<sup>2</sup> reported that marked changes in depot size induced by starvation or experimental obesity produced no change in total number of adipocytes in the epididymal and retroperitoneal depots of rats even when these alterations occurred as early as 6 weeks of age. These and other studies<sup>3-5</sup> suggest that it is the caloric intake between birth and weaning rather than later in life that plays the important role in establishing the cellularity of the adipose tissue depots of the adult. But we report here data which indicate that a reduction in food intake can markedly influence the number of adipocytes in the depots of adults even when initiated as late as 6 weeks of age.

Barrier-bred SPF Fisher 344 rats (28 d old) (Charles River) were kept in a barrier facility. All rats were initially fed Ralston-Purina diet 5755. Body weight and food consumption were recorded for 2 weeks after which the diet allotment for half the rats was reduced over 1 week to 60% that of *ad libitum* rats. Rats on the restricted food intake received diet 5755-4 (Ralston Purina) which was identical to 5755 except the vitamin content was increased so that vitamin consumption was the same in both groups of rats. Thus of the 20 rats killed at 6 months of age, 10 were fed *ad libitum* for their entire life and the other 10 were kept on the restricted food intake from 6 weeks to 6 months of age.

At 6 weeks of age, the 10 rats to be fed *ad libitum* throughout their life (*ad libitum* rats) averaged  $93 \pm 2.7$  g (s.e.m.) and the 10 rats to be fed a restricted amount of food from that time on (*restricted rats*) averaged  $96 \pm 5.7$  g. At 6 months of age the *ad libitum* rats averaged  $344 \pm 8.1$  g and the restricted rats  $212 \pm 4.2$  g.

Table 1 Left epididymal and perirenal adipose depot masses in *ad libitum* and restricted rats

| Adipose depot | Total depot mass (g)*  |                        | Depot mass (g) per total body mass (kg)* |                        |
|---------------|------------------------|------------------------|------------------------------------------|------------------------|
|               | <i>Ad libitum</i> rats | Restricted rats        | <i>Ad libitum</i> rats                   | Restricted rats        |
| Epididymal    | $5.0 \pm 0.25$         | $1.7 \pm 0.16^\dagger$ | $14.6 \pm 0.74$                          | $7.9 \pm 0.74^\dagger$ |
| Perirenal     | $5.1 \pm 0.21$         | $1.5 \pm 0.17^\dagger$ | $14.7 \pm 0.61$                          | $7.3 \pm 0.77^\dagger$ |

Rats were killed by decapitation and perirenal and epididymal fat depots were removed and placed in 0.9% NaCl at 37 °C. Adipocyte size and cell number per depot were determined by a method modified from Stiles *et al.*<sup>6</sup>, using type IV collagenase (Worthington lot 45M181X, 190 units mg<sup>-1</sup>). Crystal violet was used to stain the nuclei of adipocytes; collagenase, stromal material and the small, more slowly floating cells were removed from adipocytes by allowing the latter to float to the surface of 15 ml digestion medium without collagenase. The infranatant was discarded and the washing of the adipocytes was repeated twice. This procedure did not influence the adipocyte size distribution when compared to the procedure of Stiles *et al.* which does not remove these contaminating materials before sizing the adipocytes.

\*Data are means of 10 depots  $\pm$  s.e.m.

†Significantly different from *ad libitum*-fed rats ( $P < 0.001$ )

The restricted rats had smaller fat pads than did their *ad libitum* counterparts (Table 1). Furthermore, expression of these data as mass of depot per kg body mass showed that adipose tissue depots of the restricted animals contributed less to body mass than depots of *ad libitum* rats.

The mean diameter of the adipocytes in the epididymal or perirenal adipose depots of the restricted rats was smaller than that of the adipocytes from the *ad libitum* rats (Table 2). The total number of adipocytes in the left epididymal and perirenal fat depots of the restricted rats was markedly lower than the number in the fat depots of the *ad libitum* rats.

The pattern of development of adipose tissue in rats is well established<sup>2,7,8</sup>. From the time of weaning until approximately 14 weeks of age, the epididymal, retroperitoneal and subcutaneous depots grow by increases in both adipocyte size and number. At about the age of 14 weeks to at least 6 months of age the cell number is nearly fixed<sup>2,6,10</sup>. Similar patterns of adipose tissue development

Table 2 Adipocyte size and number of adipocytes in left epididymal and perirenal adipose depots

|                                         | <i>Ad libitum</i> rats | Restricted rats        |
|-----------------------------------------|------------------------|------------------------|
| Adipocyte diameter ( $\mu$ m)*          |                        |                        |
| Epididymal                              | $107 \pm 2.4$          | $84 \pm 3.0^\dagger$   |
| Perirenal                               | $114 \pm 2.6$          | $86 \pm 4.0^\dagger$   |
| Adipocytes ( $\times 10^6$ ) per depot* |                        |                        |
| Epididymal                              | $7.4 \pm 0.38$         | $4.6 \pm 0.19^\dagger$ |
| Perirenal                               | $6.1 \pm 0.27$         | $3.9 \pm 0.17^\dagger$ |

\*Data are means of 10 depots  $\pm$  s.e.m.

†Significantly different from *ad libitum*-fed rats ( $P < 0.001$ )

have been observed in many but not all species studied<sup>1,11,12</sup>.

Although the number of adipocytes in the depots increases well past weaning, it is generally held that the number found in depots of the adult rat can be influenced by modifying caloric intake only if initiated in the preweaning period<sup>1-5</sup>. On the basis of the studies with rats and on data obtained with man, it has been suggested that the time between birth and 2 yr of age is critical for the development of human adipose tissue and in the pathogenesis of human obesity<sup>13</sup> of human obesity<sup>13</sup>.

Although caloric restriction of adults has not been shown to influence the number of adipocytes in their depots, some nutritional manipulations in adults do influence adipocyte number. High-fat diets increase the number of adipocytes in the depots when fed to adult rats in the presence of adequate amounts of B vitamins<sup>14-16</sup>.

Our results show that the number of adipocytes in the depots of adult rats can be markedly reduced by food restriction initiated 2 weeks after weaning. The preweaning period is thus not the only period sensitive to reduction in food intake in regard to establishing adipocyte number in adult depots. How much later in childhood this nutritional regime can be initiated and still have a sizeable effect on adipose tissue cellularity is of great interest, but the design of our ageing research programme precluded obtaining such data. Whether food restriction initiated at 6 weeks of age leads to permanent reduction in adipocyte number (that is, one not reversed by *ad libitum* feeding), as is the case with preweaning restriction, was also not explored. It is possible that control of the

development of adipose tissue cellularity may well be in the province of the paediatrician's work with schoolage children.

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## Proline analogue removes fibroblasts from cultured mixed cell populations

PRIMARY cultures of cells from various tissues have become important tools for many experimental studies, but attempts to prepare primary cultures of specialised cell types are frequently frustrated by the tendency of fibroblasts to grow more rapidly than other cells. During studies on procollagen biosynthesis by fibroblasts, it has been found that if the cells are incubated with any one of four proline analogues, the analogues are incorporated into protein and the incorporation of the analogues into procollagen polypeptides prevents the chains from folding into a triple helical conformation<sup>1-7</sup>. We report here that if fibroblasts are grown in the presence of the proline analogue *cis*-hydroxyproline, their rate of growth is markedly limited. This may provide a simple technique for removing fibroblasts from cultures of mixed cell populations.

Fibroblasts isolated by controlled enzymatic digestion of the tendons of 17-d-old chick embryos, and primary cultures were prepared as described before<sup>8</sup>. Addition of 100  $\mu\text{g ml}^{-1}$  of *cis*-hydroxyproline (*cis*-4-hydroxy-L-proline or allohydroxy-L-proline; Ajinomoto) to logphase cultures decreased the growth rate within 24 h (Fig. 1a) and thereafter the number of cells per flask decreased to less than the initial level. The same concentration of *cis*-hydroxyproline had no effect on the growth of KB cells (Fig. 1b), a cultured line of cells developed from a human epidermoid carcinoma. Also, it had no effect on the growth of a neuroblastoma cell line (Fig. 1c).

Examination of varying concentrations of *cis*-hydroxyproline (Fig. 2) demonstrated that the growth of primary cultures of tendon cells in late-log phase was inhibited with 50  $\mu\text{g ml}^{-1}$  or more of *cis*-hydroxyproline. Cultures of mouse L-929 fibroblasts were more resistant and a concentration of 100-200  $\mu\text{g ml}^{-1}$  was required to inhibit the growth. The growth of neuroblastoma or KB cells was not affected with 200  $\mu\text{g ml}^{-1}$ , but there was partial inhibition with higher concentrations. The sensitivity to *cis*-hydroxyproline was not related in any simple manner to the rate of cell growth; in the experiments reported here the doubling time was 16 h for the primary cultures of tendon fibroblasts, 30 h for the L-929 fibroblasts, 24 h for the KB cells, and 24 h for the neuroblastoma cells (Fig. 1).

The selective effect of *cis*-hydroxyproline on fibroblasts was further illustrated by preparing mixed cultures of fibroblasts and KB cells, and then labelling the cultures with antibodies specific for prolyl hydroxylase<sup>9</sup>, the enzyme which synthesises the hydroxyproline in procollagen. The antibody was conjugated with peroxidase and located in cells by reacting the peroxidase with 3,3'-diaminobenzidine<sup>10</sup> (Sigma). Figure 3a shows that fibroblasts were readily distinguished from KB cells in untreated cultures. In cultures treated with 100  $\mu\text{g ml}^{-1}$  of *cis*-hydroxyproline for 7 d, KB cells were present but no fibroblasts were discernible either by morphology or a specific antibody stain for prolyl hydroxylase (Fig. 3b).

In a separate series of experiments, cultures in late log phase were incubated with <sup>14</sup>C-proline and then assayed for non-dialysable <sup>14</sup>C-hydroxyproline<sup>11</sup>, as a measure of the rate of procollagen synthesis. Cultures which were the most sensitive to *cis*-hydroxyproline (Fig. 2) had the highest rate of procollagen synthesis (Table 1). Also, after mixed cultures of tendon fibroblasts and KB cells were grown in the presence of *cis*-hydroxyproline for 5 weeks, there was

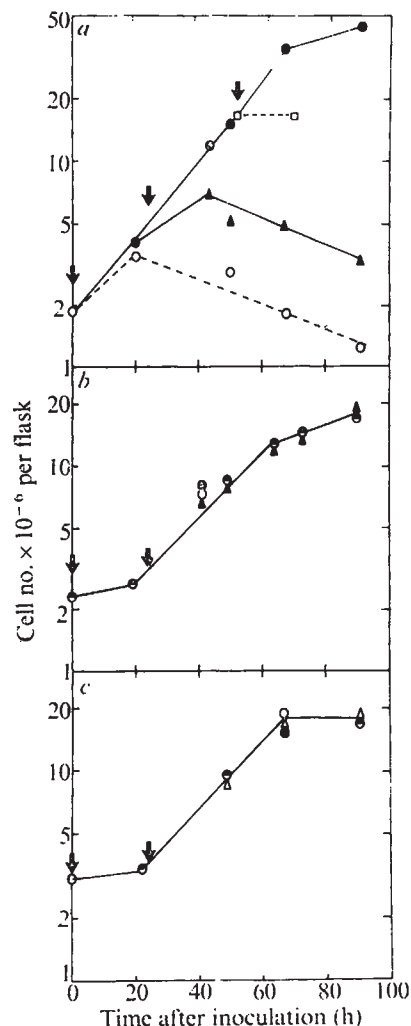


Fig. 1 Effects of *cis*-hydroxyproline on the growth of a, tendon fibroblasts; b, KB cells and c, neuroblastoma cells. Tendon fibroblasts were obtained from limited enzymatic digestion of tendon from 17-d-old chick embryos<sup>8</sup>. KB cells (CCL-17) and neuroblastoma cells (CCL-131) were from the American Type Culture Collection. The cells were grown in an atmosphere of 10% CO<sub>2</sub> and 90% air in Dulbecco's medium supplemented with 10% foetal calf serum, 100 units ml<sup>-1</sup> of penicillin, and 100  $\mu\text{g ml}^{-1}$  of streptomycin. The culture media were changed every other day after inoculation. *cis*-Hydroxyproline (100  $\mu\text{g ml}^{-1}$ ) was added to cultures at time indicated by arrow. Cell number per flask (Falcon; 75 cm<sup>2</sup>) was estimated with a haemocytometer. Values presented are the mean from duplicate cultures. Cultures were grown in control conditions (●), or with *cis*-hydroxyproline added at time of inoculation (○), 24 h after inoculation (▲), or 54 h after inoculation (□).

no apparent recovery of procollagen synthesis when the cells were subsequently subcultured without *cis*-hydroxyproline for 1 week. Morphologically the cells were similar to KB cells. These results suggest that the fibroblasts were effectively removed from the mixed cultures.

The effect of *cis*-hydroxyproline on the growth of fibroblasts in culture might be explained by the previous observations indicating that when cells are incubated with proline



**Table 1** Effect of *cis*-hydroxyproline on synthesis of non-dialysable  $^{14}\text{C}$ -hydroxyproline by cell cultures

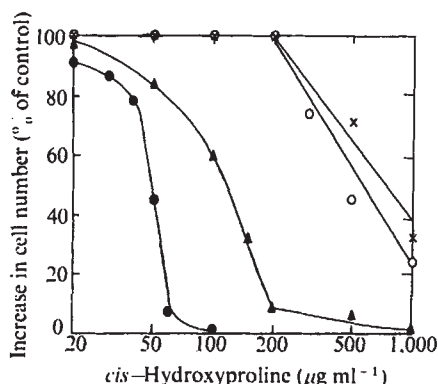
| Cells                         | Treatment                           | $^{14}\text{C}$ -hydroxyproline synthesised (d.p.m. per $10^7$ cells per 30 min) |
|-------------------------------|-------------------------------------|----------------------------------------------------------------------------------|
| Tendon fibroblasts            |                                     | 5,691                                                                            |
| L-929 fibroblasts             |                                     | 844                                                                              |
| KB cells                      |                                     | < 100                                                                            |
| Neuroblastoma                 |                                     | < 100                                                                            |
| Tendon fibroblasts            |                                     | 4,974                                                                            |
| Tendon fibroblasts + KB cells | + cHyp, 6 weeks*                    | < 100                                                                            |
| Tendon fibroblasts + KB cells | + cHyp, 5 weeks;<br>- cHyp, 1 week† | < 100                                                                            |

For labelling, medium was removed from each flask that contained  $2.5 \times 10^7$  cells on the 6th day after the last subculturing and fresh medium (without *cis*-hydroxyproline) containing  $1 \mu\text{Ci ml}^{-1}$  of  $^{14}\text{C}$ -proline was added (10 ml per flask) as described before<sup>9</sup>. In the second experiment, labelling period was 4 h instead of 30 min and values are reduced proportionally to make them comparable to the first experiment. Assay did not permit detection of less than 100 d.p.m. of  $^{14}\text{C}$ -hydroxyproline.

\* Mixed cultures (1:1) of tendon fibroblasts from chick embryos and KB cells were incubated continuously with  $100 \mu\text{g ml}^{-1}$  of *cis*-hydroxyproline (cHyp) through 6 subcultures over 6 weeks.

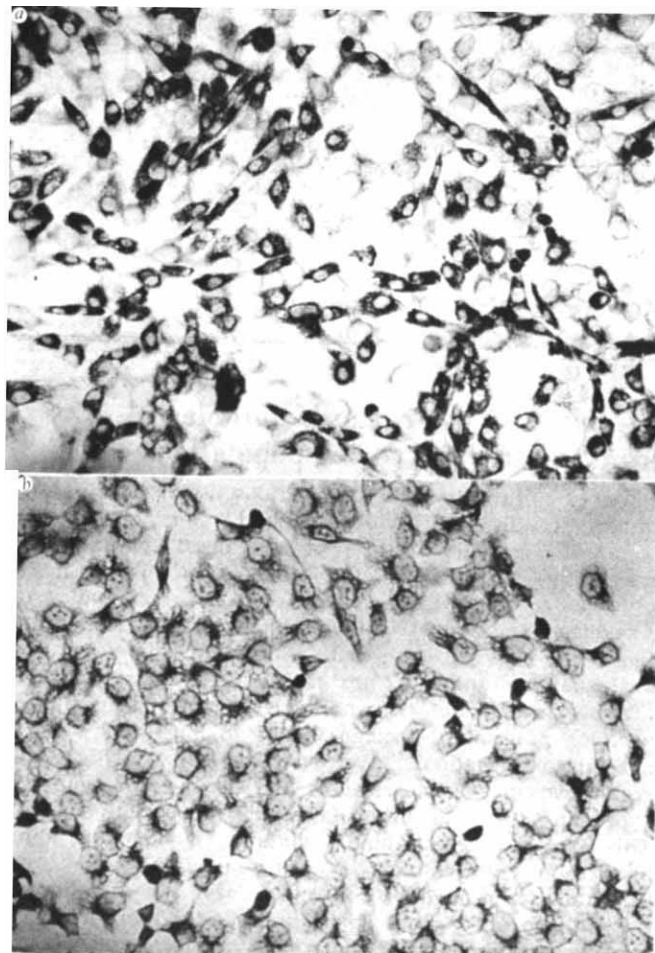
† Mixed cultures (1:1) were incubated continuously with  $100 \mu\text{g ml}^{-1}$  of *cis*-hydroxyproline through 5 subcultures over 5 weeks and then for a 6th subculture without the analogue for 1 week.

analogues, the analogues are incorporated into procollagen and the procollagen containing the analogues cannot become triple helical<sup>1-7</sup>. Several observations have suggested that the secretion and deposition of triple-helical collagen on surfaces used to culture cells may enhance the rate of growth of some fibroblasts *in vitro*<sup>8,12</sup>. It is possible therefore that *cis*-hydroxyproline limits the rate of fibroblast growth in the conditions employed here because it prevents the deposition of triple-helical collagen on the cell layer. This hypothesis, however, has not been explored



**Fig. 2** Dose dependency of effect of *cis*-hydroxyproline on the growth of cells. Various amounts of *cis*-hydroxyproline were added to cultures at mid-log phase about 48 h after inoculation. In each case the experiment was terminated after the cell number in control cultures had doubled. Cells used were tendon fibroblasts (●); L-929 fibroblasts (▲); KB cells (○); and neuroblastoma cells (X).

in detail and it may well be that *cis*-hydroxyproline decreases the growth of fibroblasts in culture by some entirely unrelated mechanism. Nevertheless, use of *cis*-hydroxyproline is a simple technique which may be useful in preventing an overgrowth of fibroblasts, thus facilitating the preparation of primary cultures of other cell types. Other proline analogues which are incorporated into protein may be useful for the same purposes, although  $0.2 \text{ mM}$  ( $25 \mu\text{g ml}^{-1}$ ) 3,4-dehydroproline was found not to inhibit the growth of L-929 fibroblasts<sup>13</sup>.



**Fig. 3** Selective removal of tendon fibroblasts by *cis*-hydroxyproline from a mixed culture of tendon fibroblasts and KB cells. Equal numbers of tendon fibroblasts and KB cells were mixed and inoculated on coverslide inside a culture tube (Leighton type, Bellco) at a density of about  $4 \times 10^4 \text{ cm}^{-2}$ . To half of the cultures  $100 \mu\text{g ml}^{-1}$  of *cis*-hydroxyproline was added at the time of inoculation. The culture medium was changed every other day, and the cultures were maintained for 1 week. The cells on cover slides were fixed with ethanol-ether mixture, and then incubated with peroxidase conjugated to antibodies directed against chick prollyl hydroxylase. The samples were stained with 3,3'-diaminobenzidine as described before<sup>9</sup>. *a*, Photomicrograph of control sample. Tendon fibroblasts appear spindle shaped and are darkly stained. *b*, Photomicrograph of mixed cultures treated with *cis*-hydroxyproline. Only KB cells are seen; the few darkly-stained bodies probably represent dead cells.

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## Prostaglandin E<sub>2</sub>-9-ketoreductase as a mediator of salt intake-related prostaglandin-renin interaction

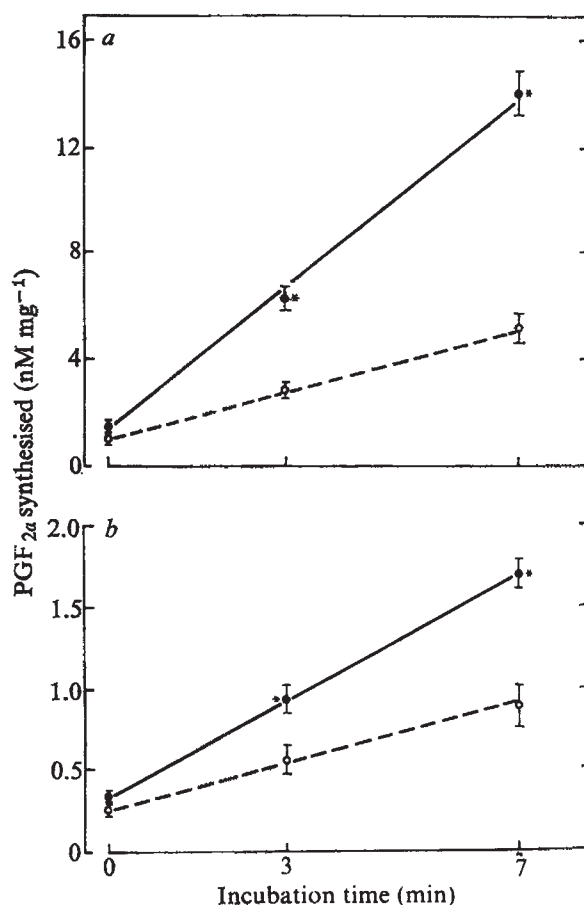
PREVIOUS studies have revealed a close relationship between the prostaglandin (PG) and the renin-angiotensin systems in the kidney<sup>1,4</sup> and we assume that both systems are intimately involved in the regulation of salt and volume homeostasis and of blood pressure. Changes in NaCl and water balance were found to be important determinants of the activity and function of the renin-angiotensin system<sup>5</sup> and it has been suggested that PG synthesis is also modified by these variables<sup>2,6,7</sup>. One factor influencing the pathway and, thus, the consequences of altered PG formation is the enzyme PGE<sub>2</sub>-9-ketoreductase, which catalyses the interconversion of the antagonistically acting PGE<sub>2</sub> and PGF<sub>2α</sub> (ref. 8). First direct evidence is presented here that PGE<sub>2</sub>-9-ketoreductase activity is under the control of NaCl ingestion. We suggest that this enzyme is an important mediator of salt intake-related prostaglandin-renin interaction.

PGE<sub>2</sub>-9-ketoreductase activity has been demonstrated in the renal cortex and medulla of several species, including man and rabbit<sup>9,10</sup>. In our investigation in the rabbit, the effects of low and high NaCl ingestion on renal cortical and medullary PGE<sub>2</sub>-9-ketoreductase and plasma renin activity were examined. Since urinary prostaglandin excretion is assumed to reflect renal prostaglandin biosynthesis<sup>11</sup>, urinary PGE<sub>2</sub> and PGF<sub>2α</sub> excretion were also measured (Table 1 and Fig. 1). At high NaCl intake, sodium excretion was nine times, but the urinary volume was only twice that at low NaCl intake. Plasma renin activity (PRA) was 12.9 ng ml<sup>-1</sup> h<sup>-1</sup> at low and decreased to 1.8 ng ml<sup>-1</sup> h<sup>-1</sup> at high NaCl intake. At low NaCl intake, urinary excretion of PGE<sub>2</sub> per day measured 1.9 µg and of PGF<sub>2α</sub> 2.1 µg, giving a ratio of PGE<sub>2</sub> to PGF<sub>2α</sub> of 1:1.1. PGE<sub>2</sub>-9-ketoreductase activity in renal cortex was 0.61 ± 0.07 nM PGF<sub>2α</sub> formed min<sup>-1</sup> mg<sup>-1</sup> (s.e.m., *n* = 13) and in the medulla 0.1 ± 0.01 (s.e.m., *n* = 13). At high NaCl intake, PGE<sub>2</sub>-9-ketoreductase activity in the renal cortex increased threefold to 1.74 ± 0.13 (s.e.m., *n* = 10; *P* < 0.001) and in the medulla twofold to 0.19 ± 0.01 (s.e.m., *n* = 10; *P* < 0.001). At the same time urinary excretion of PGE<sub>2</sub> per day decreased to 0.53 µg, whereas that of PGF<sub>2α</sub> remained practically unchanged at 2.43 µg, giving a ratio of PGE<sub>2</sub> to PGF<sub>2α</sub> of 1:4.6.

The observed decrease in urinary PGE<sub>2</sub> excretion on high NaCl intake may result from either a decrease in renal PGE<sub>2</sub> synthesis, an alteration in PGE<sub>2</sub> metabolism at maintained synthesis, or both. The total amount of PGE<sub>2</sub> and PGF<sub>2α</sub> excreted in urine decreased at high NaCl intake, suggesting a lower renal PG production. A reduction in formation or breakdown of PG endoperoxides, however, could only explain the lower PGE<sub>2</sub> excretion but not account for the unchanged PGF<sub>2α</sub> excretion found on high NaCl intake. These findings can best be interpreted in terms of an altered pathway of PGE<sub>2</sub> metabolism on reduced PG synthesis. The increase of renal cortical and medullary PGE<sub>2</sub>-9-ketoreductase activity, as demonstrated in this study during high NaCl intake, shifts intrarenal PG formation from PGE<sub>2</sub> to PGF<sub>2α</sub>, and could therefore explain the decrease in urinary PGE<sub>2</sub> excretion while PGF<sub>2α</sub> excretion remains unchanged. Such a mechanism may be operative in man, since it has been observed in disease states in which variations in sodium and volume homeostasis are accompanied by changes in the PGE<sub>2</sub> to PGF<sub>2α</sub> ratio<sup>12</sup>.

The decrease of PRA after high NaCl intake confirms earlier observations<sup>5</sup> and reflects, in these conditions, a decrease in renin activity within the juxtaglomerular apparatus, which regulates synthesis and release of this enzyme. *In vitro*, renin release is increased by the PG precursor arachidonic acid, by PG endoperoxides or by PGE<sub>2</sub>, whereas PGF<sub>2α</sub> inhibits renin secretion<sup>13,14</sup>. PGF<sub>2α</sub> may have this action also *in vivo*. Therefore, the decrease in PRA during salt loading could result from the less opposed inhibitory action of PGF<sub>2α</sub> on renin release. This assumption is supported by recent findings in man and dog, in

which renin release decreased concomitant with increased sodium and PGF<sub>2α</sub> excretion<sup>15,16</sup>. Thus, at high NaCl intake, the sodium-conserving renin-angiotensin system may be blocked by the relative increase in PGF<sub>2α</sub> on reduced PG synthesis. In addition, distal nephron action of the antidiuretic hormone may be unopposed as a result of decreased PGE<sub>2</sub> formation<sup>17,18</sup>. These mechanisms would, at high NaCl intake, facilitate a high sodium load to be eliminated in a small urine volume, as demonstrated in our experiments (ninefold increase in urinary sodium excretion compared with twofold increase in urinary volume). At low NaCl intake the reverse was observed: PG synthesis may increase. PGE<sub>2</sub>-9-ketoreductase activity is reduced, thereby increasing PGE<sub>2</sub> levels. Because of the stimulatory and relatively less opposed



**Fig. 1** Enzyme kinetic determinations of renal cortical (a) and medullary (b) PGE<sub>2</sub>-9-ketoreductase activity of rabbits at low (O) and high (●) NaCl intake, respectively. Mean values are given as nM PGF<sub>2α</sub> formed per mg protein (± s.e.m. *n* = 13 for low, *n* = 10 for high NaCl intake). NADPH-dependent PGE<sub>2</sub>-9-ketoreductase activity was determined by the method of Stone and Hart<sup>9</sup> with minor modifications. Cortex and medulla of the removed kidneys were dissected at 4 °C. A 20% homogenate was prepared in 9.2 mM potassium phosphate buffer (pH 7.3) at a final concentration of 4 mM MgCl<sub>2</sub> and 0.1 mM 1,4-dithioerythritol (Merck, W. G.). The suspensions of cortex and medulla were centrifuged for 60 min at 100,000 *g*. The high speed supernatant fluids were dialysed for 24 h against 100 volumes phosphate buffer. Incubations were carried out at 37 °C using 200 µl supernatant as the source of PGE<sub>2</sub>-9-ketoreductase, in a total volume of 600 µl phosphate buffer, at final concentrations of 1 mM NADPH<sub>2</sub> (Merck, W. G.) as cofactor and 1.2 mM PGE<sub>2</sub> (Upjohn, Kalamazoo) as substrate in excess (≥ fourfold *K<sub>m</sub>*). At 0, 3 and 7 min, 100 µl of the mixture were removed. The reaction was stopped by adding 500 µl ice-cold Tris-HCl buffer (10 mM, pH 7.4, containing 0.1% gelatin and 140 mM NaCl), and heating the samples for 2 min to 100 °C. The amounts of PGF<sub>2α</sub> enzymatically converted from PGE<sub>2</sub>, were analysed by RIA (ref. 9). Reaction velocity of PGE<sub>2</sub>-9-ketoreductase was calculated from the amounts of PGF<sub>2α</sub> generated per minute and per milligram protein, determined by the method of Lowry *et al.* (ref. 25).

\* *P* < 0.001. Level of significance between values obtained in rabbits on low and high NaCl diet was determined by the unpaired Student's *t* test.



**Table 1** Effects of low and high NaCl intake on renal parameters in rabbits

| NaCl intake<br>(per 100 g food) | UV <sub>Na</sub> <sup>+</sup><br>(mmol d <sup>-1</sup> ) | UV<br>(ml d <sup>-1</sup> ) | Urinary PGE <sub>2</sub><br>excretion<br>(µg d <sup>-1</sup> ) | Urinary PGF <sub>2α</sub><br>excretion<br>(µg d <sup>-1</sup> ) | PRA<br>(ng ml <sup>-1</sup> h <sup>-1</sup> ) |
|---------------------------------|----------------------------------------------------------|-----------------------------|----------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------|
| Low: (13) 0.25 g                | 6.1 ± 0.4                                                | 146 ± 8                     | 1.9 ± 0.1                                                      | 2.1 ± 0.2                                                       | 12.9 ± 1.2                                    |
| High: (10) 2.50 g               | 53.8 ± 4*                                                | 295 ± 11*                   | 0.53 ± 0.17*                                                   | 2.43 ± 0.3                                                      | 1.8 ± 0.3*                                    |

The effects of a sodium-depleted (0.25 g NaCl per 100 g food; or sodium-enriched (2.5 g NaCl per 100 g food) diet (Altromin, W. G.) on daily urinary sodium excretion (UV<sub>Na</sub>) and urinary volume (UV), PGE<sub>2</sub> and PGF<sub>2α</sub> excretion and PRA in cross-bred rabbits. Values are given as mean ± s.e.m. Numbers in parentheses indicate the number of animals. Experiments were carried out in rabbits kept in metabolic conditions for 10 d on either diet with drinking water *ad libitum*. Urinary parameters and PRA were measured in conscious animals the day before the kidneys were removed under pentobarbital anaesthesia for determination of renal PGE<sub>2</sub>-9-ketoreductase activity. PRA was determined by radioimmunoassay (RIA) of angiotensin I as described previously<sup>23</sup>. PGE<sub>2</sub> and PGF<sub>2α</sub> were analysed after previous extraction and group separation of the urinary PGs on silicic acid columns (Unisil, Clarkson Chemicals, Pennsylvania)<sup>11,12</sup>. PGE<sub>2</sub> was measured in parallel by bioassay of PGE<sub>2</sub> (ref. 24) and by RIA using a PGB-specific antibody after conversion of the PGE fraction to PGB<sup>12</sup>. The correlation between values obtained with both methods was highly significant ( $y = 0.8x + 4.9$ ;  $r = 0.94$ ). PGF<sub>2α</sub> excretion was determined using a commercially available PGF<sub>2α</sub> antibody (Calbiochem, Pennsylvania)<sup>11,12</sup>.

\*  $P < 0.001$ . Level of significance between values obtained in rabbits on a low and high NaCl diet was determined by the unpaired Student's *t* test.

action of PG endoperoxides or PGE<sub>2</sub> on renin release, the stimulated renin activity could then initiate the sodium-conserving mechanism<sup>5</sup>. In addition, at low NaCl intake, increasing PGE<sub>2</sub> levels may enhance distal tubular sodium reabsorption<sup>18</sup>, and further support the effectiveness of the stimulated sodium-conserving renin-angiotensin system.

We therefore propose the concept that the activity of renal PGE<sub>2</sub>-9-ketoreductase is dependent on NaCl ingestion or some factor related to salt intake. Our results suggest that this enzyme is intimately involved in adjusting the activity of the renin-angiotensin system and distal nephron function in response to varying levels of NaCl intake. Thus, renal PGE<sub>2</sub>-9-ketoreductase may play a pivotal role in the regulation of NaCl and water balance. Finally, the demonstration of PG synthesis and of PGE<sub>2</sub>-9-ketoreductase activity in blood vessel walls<sup>19,20</sup> may provoke new speculations on the finding that vascular reactivity and blood pressure are influenced by the state of NaCl balance (refs 21 and 22).

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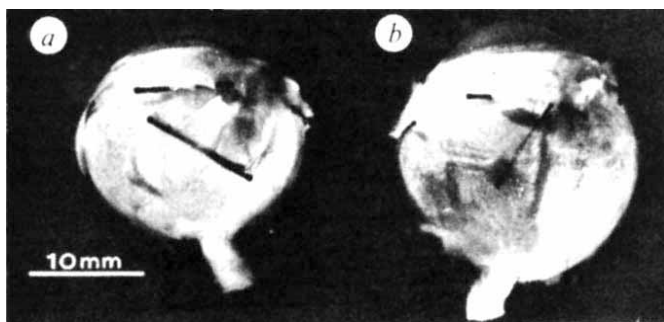
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lenses. The mechanism of myopia can be rationally analysed only if a suitable animal model is found for this refractive condition. In the literature, there are only two examples of experimental myopia. Levinsohn<sup>2</sup> claimed that a high degree of myopia develops in young monkeys when the body is kept elevated above the head for long periods of time; his results, however, have been questioned<sup>3,4</sup>. Young<sup>5</sup> induced myopia by exposing monkeys to a restricted visual space, but the refractive error thus obtained was relatively small. During investigations on monocular visual deprivation in monkeys<sup>6</sup> it was noted that the eye in which the lids had been closed by suture at birth developed a high degree of myopia. We report here a study of the effects of neonatal lid fusion on the refractive state and anatomy of the macaque monkey eye.

In 10 macaque monkeys (*Macaca mulatta*, *Macaca arctoides*) monocular or binocular lid fusion was performed at various times after birth. The surgical technique was as follows: under general anaesthesia, the margin of both lids was excised lateral to the lacrimal papilla. The conjunctiva was removed for an additional couple of millimetres to prevent its ingrowth into the wound and the cut surfaces of the lids were approximated by silk mattress sutures from the lateral canthus to the lacrimal papilla. Sutures were removed 1–2 weeks after surgery, when the lids had fused and appeared as a thin, translucent diaphragm that attenuated light by about half a log unit. After a variable period of lid fusion (19 d to 26 months) the palpebral fissure was re-established. Accommodation was paralysed with 2 drops



**Fig. 1** Eyes of a rhesus monkey in which the lids on the right (b) were fused at the age of 2 weeks and opened 18 months later (experiment 5 in Table 1). Suture threads mark the insertions of the extrinsic ocular muscles. The left eye (a) was normal.

## Myopia and eye enlargement after neonatal lid fusion in monkeys

THE aetiology of myopia has been studied mainly by investigating the distribution of refractive errors in human populations<sup>1</sup>. No clear conclusion has emerged, however, so the prevailing clinical attitude is that myopia can neither be prevented nor cured, but only corrected with appropriate

of a 1% solution of homatropine and the refraction of both eyes determined by using a streak retinoscope and hand-held trial case lenses. The corneal curvature was measured with a keratometer and the fundus was examined. At various times after lid opening, animals were refracted again, used for electrophysiological studies on the visual pathways and finally perfused through the heart with 10%

**Table 1** Interocular difference in refraction and axial length following neonatal lid fusion in macaque monkeys

| Experiment no. | Age at lid fusion | Duration of lid fusion | Difference in refraction (dioptré) | Difference in axial length* | <i>Macaca</i> species |
|----------------|-------------------|------------------------|------------------------------------|-----------------------------|-----------------------|
| 1              | 11 d              | 19 d                   | -1.0                               | —                           | <i>mulatta</i>        |
| 2              | Birth             | 6 weeks                | -2.75                              | 1.089                       | <i>mulatta</i>        |
| 3              | 11 d              | 6 months               | -7.0                               | 1.077                       | <i>mulatta</i>        |
| 4              | 4 d               | 12 months              | -9.5                               | 1.101                       | <i>arctoides</i>      |
| 5              | 2 weeks           | 18 months              | -13.5                              | 1.208                       | <i>mulatta</i>        |
| 6              | 6 weeks           | 15 months              | -8.5                               | 1.108                       | <i>mulatta</i>        |
| 7              | 12 months         | 26 months              | -4.5                               | 1.010                       | <i>arctoides</i>      |
| 8              | mature            | 17 months              | 0                                  | —                           | <i>mulatta</i>        |

\*Expressed as a ratio between axial length of the myopic eye and axial length of the normal eye.

formalin. The eyes were enucleated, measured and processed for light microscopy; in some animals, the extra-ocular muscles were prepared for microscopy.

To illustrate the effects of monocular lid fusion on the refraction and the anatomy of the eye, we describe an experiment in which results were striking and a comprehensive morphological analysis was performed (experiment 5 in Table 1). Lids were sutured in the right eye of a rhesus monkey at the age of 2 weeks and the fusion maintained for 18 months. Upon re-establishing the palpebral fissure, a normal conjunctival sac was found and the appearance of the exposed surface of the eye globe was identical to the control side. The interocular difference in curvature of the anterior corneal surface was slight (average refractive power in the right eye 51 dioptres, in the left eye 52.5 dioptres). Retinoscopy showed that the dioptric media were normally transparent and that the right eye was 13.5 dioptres more myopic than the left eye. The appearance of the fundus was normal; furthermore, the physiological experiments indicated that the lateral geniculate bodies received normal retinal input from both eyes.

At autopsy, the right eye was considerably larger than the left eye (Fig. 1). Various eye dimensions were measured and some of the results are diagrammatically represented in Fig. 2. The most remarkable finding was a 21% increase in the axial length of the right eye, as measured from the summit of the cornea to the projection of the fovea on the sclera (corresponding to the point of entry of the lateral long posterior ciliary artery and long ciliary nerve into the eye globe); the increase in equatorial diameter of the right eye was 7% only. Corneal diameters and distance of the insertions of the rectus muscles from the limbus were identical in both eyes, whereas the linear distance of the muscle insertions from the optic nerve was 23% larger in the right eye. Finally, the distance of the fovea from the centre of the optic disk was 18% larger in the myopic eye.

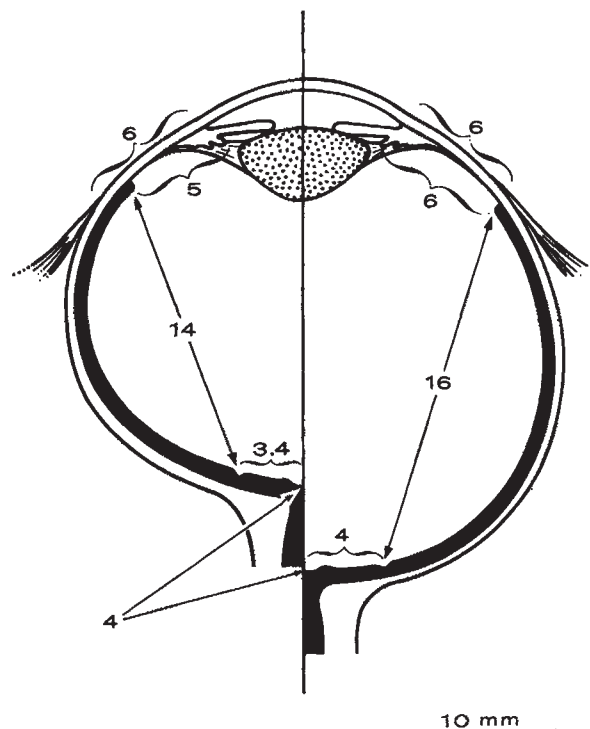
Histological examination showed that the posterior sclera was thinner in the right eye. Thickness of the cornea, choroid and retina were identical in both eyes. Also identical were the cross-sectional area of the ciliary muscle, the diameter of the optic nerve, the structure of the optic nerve head, and the cross-sectional area of the extrinsic ocular muscles.

Thus, neonatal lid fusion caused an elongation of the eye globe, characterised by distension of the postequatorial hemisphere and attenuation of the posterior sclera. All remaining ocular structures seemed unchanged.

Table 1 lists the results of all of our monocular lid fusion experiments. It shows that the degree of myopia caused by lid fusion increased with the duration of closure, that the error of refraction was less pronounced when lids were fused in older animals, and that there was no change in the adult. It should be noted that there was some variability in the response of different individuals to lid fusion, but

that all animals in which lids were fused early in life became myopic.

In order to establish whether the refractive error was caused by a functional imbalance between the eyes, lids were fused bilaterally in a rhesus monkey at the age of 5 weeks. Eleven months later, -11-dioptre myopia was present bilaterally and both eyes were equally enlarged. In



**Fig. 2** Diagram illustrating the effects of neonatal lid fusion on various eye dimensions. The temporal halves of the eyes are juxtaposed. (Experiment 5 in Table 1.)

another instance, lids were fused in the right eye of a rhesus monkey at the age of 1 week and opened 12 months later; a -10-dioptre myopia was present in the sutured eye. Lids were subsequently fused in the left eye and opened 13 months later; this eye only developed a -6-dioptre myopia. The axial length was 23.25 mm in the right eye and 22 mm in the left eye (the average axial length of the eye in the mature rhesus monkey was 19.34 mm  $\pm$  0.14 s.e.m., measured in 15 animals, right and left eyes pooled). These experiments confirm that larger refractive errors are obtained in younger animals and demonstrate that the development of myopia in each eye is independent of the functional state of the other eye. Finally, it is important to mention that myopia persists unmodified for years after lid opening.

The myopia caused by lid fusion is clearly of the axial type, for the corneal refraction is not affected significantly.



The fact that the refractive error is larger when lid fusion is performed early in life, suggests that this type of myopia represents a derangement of the development of the eye. Presumably, neonatal lid fusion somehow accelerates and accentuates a mechanism which operates normally in post-natal life. Supporting this interpretation is the finding that the postequatorial segment of the globe elongates, whereas the anterior segment is relatively unaffected; in fact, it is known that in humans the cornea changes but little after birth, whereas the axial length increases dramatically<sup>1</sup>. The simplicity and effectiveness of the technique and the fact that one eye can be used as a control, indicate to us that monocular myopia caused by neonatal lid fusion in monkeys represents a useful animal model for myopia in general and that a study of its pathogenesis may provide insight into the development of this refractive error in humans. After a presentation of our results<sup>2</sup>, G. K. van Noorden reported inconsistent changes in refraction following lid fusion in neonatal rhesus monkeys; we have no explanation for the discrepancy.

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## Remyelination of CNS axons by Schwann cells transplanted from the sciatic nerve

SCHWANN cells can remyelinate axons normally myelinated by oligodendrocytes, after either primary demyelination<sup>1–3</sup> or suppression of myelination by X irradiation at the time of myelination<sup>4–5</sup>. It has been suggested that the glial limiting membrane normally inhibits Schwann cell invasion of the CNS (ref. 2) and its reformation inhibits uncontrolled invasion by Schwann cells once this phenomenon has been initiated<sup>1–2</sup>. The Schwann cells in these situations are thought to arise from the nerve roots. I undertook experiments to determine whether it is possible to suppress remyelination following primary demyelination in an experimental animal. If so the resulting demyelinated axons would be similar to those present in the multiple sclerosis plaque, that is they would remain demyelinated unrepaired by glial or Schwann cells. Using such a lesion it would then be possible to establish if Schwann cells obtained from a source outside the central neuraxis would remyelinate these axons. In the past, attempts at transplanting peripheral nervous tissue into the central nervous system have shown very limited invasion of peripheral elements into the neuropil of the CNS (refs. 6, 7) and it was hoped that the presence of large numbers of demyelinated axons would act as a stimulus for Schwann cell invasion<sup>8</sup>. In addition, intraspinal injections of lysolecithin facilitate Schwann cell invasion of the CNS from local sources<sup>2–9</sup>. By abolishing this intrinsic Schwann cell remyelination potential and by substituting an extrinsic source of Schwann cells it

might be possible to determine if persistently demyelinated axons in the CNS, similar to those in multiple sclerosis plaques, can be repaired by outside interference. I report here the preparation of areas of demyelination in rats and cats which remyelinated only in the presence of added Schwann cells.

The first step was to induce an area of primary demyelination which was incapable of being remyelinated with the cells available within the central neuraxis, that is, by oligodendrocytes or by Schwann cells derived from the spinal roots.

Intraspinal injection of lysolecithin (lysophosphatidyl choline) induces an area of primary demyelination at the site of injection in rats and cats<sup>2–9</sup> and all the demyelinated axons are subsequently remyelinated either by oligodendrocytes or Schwann cells depending on their location within the lesion. In order to suppress this remyelination the spinal cord caudal to Th<sub>10</sub> spinal level was irradiated 2 weeks before the injection of lysolecithin with 4,000 rad of X rays (from a Marconi radiotherapy machine using 250 kV, 15 mA, 0.5 mm Cu, 1 mm Al, f.s.d. 50 cm for cats and 32 cm for rats, under pentobarbitone anaesthesia). In rats and cats subjected to this procedure there was no evidence of remyelination when they were subsequently killed (Table 1 and Fig. 1). These experiments therefore established that X irradiation with 4,000 rad before demyelination suppresses both oligodendrocyte and Schwann cell remyelination, leaving large numbers of persistently demyelinated axons in the spinal cord (Fig. 1).

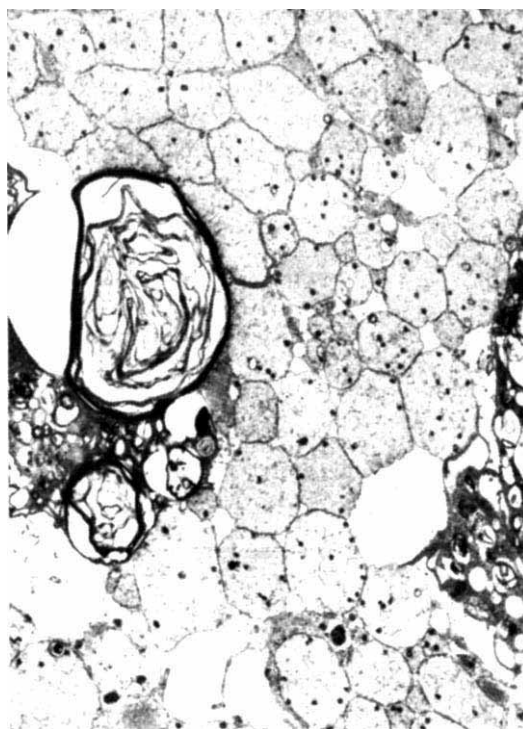


Fig. 1 Persistently demyelinated axons in the dorsal column following lysolecithin injection into the X-irradiated spinal cord (rat R53). ( $\times 4,400$ .)

In the second series of experiments with these two species a 1.0-cm length of dissociated sciatic nerve was placed over the injected area to provide a source of viable Schwann cells. The experimental procedure was as follows. At the time of spinal cord irradiation the cat or rat sciatic nerve was crushed at the mid-thigh level. A laminectomy was carried out 2 weeks later in the region of Th<sub>12–13</sub>, the dura were incised and lysolecithin was injected into the dorsal column as before<sup>2–9</sup>. In most cases 3–5  $\mu$ l of a 1.0% solution of lysolecithin (Sigma) in saline were injected in up to three sites using a hand held glass micropipette attached to an Aglar syringe (Wellcome reagents) by means of fine polythene tubing. A 1.0-cm length of sciatic nerve was then excised distal to the previously made crush, desheathed and slightly teased in tissue culture media

Table 1 Relationship between experimental treatment and remyelination

| Animal | Experimental treatment |             |                 |               | Type of remyelination          |                            |                          | Comments                                 |
|--------|------------------------|-------------|-----------------|---------------|--------------------------------|----------------------------|--------------------------|------------------------------------------|
|        | Lyso-lecithin          | Irradiation | Transplantation | Survival time | Oligo-dendrocyte remyelination | Schwann cell remyelination | Persistent demyelination |                                          |
| Rats   |                        |             |                 |               |                                |                            |                          |                                          |
| R 68   | T                      | O           | O               | 7 W           | +++                            | +                          | —                        | Small deep lesion                        |
| R 69   | T                      | O           | O               | 7 W           | ++                             | +++                        | —                        | Several large lesions                    |
| R 52   | O                      | T           | O               | 7 W           | —                              | —                          | —                        | No abnormalities                         |
| R 53   | T                      | T           | O               | 18 d          | —                              | —                          | +++                      |                                          |
| R 54   | T                      | T           | O               | 27 d          | —                              | —                          | +++                      |                                          |
| R 55   | T                      | T           | O               | 19 d          | —                              | —                          | +++                      |                                          |
| R109   | T                      | T           | T               | 33 d          | —                              | +++                        | +                        | Ventral lesion persistent demyelination  |
| R110   | T                      | T           | T               | 24 d          | —                              | +++                        | +                        | Ventral lesion persistent demyelination  |
| R111   | T                      | T           | T               | 24 d          | —                              | +++                        | +                        |                                          |
| R113   | T                      | T           | T               | 33 d          | —                              | +                          | +                        | Very small lesion                        |
| Cats   |                        |             |                 |               |                                |                            |                          |                                          |
| R108   | T                      | T           | O               | 20 d          | —                              | —                          | +++                      |                                          |
| R128   | T                      | T           | O               | 7 W           | —                              | —                          | +++                      |                                          |
| R132   | T                      | T           | O               | 8 W           | —                              | —                          | +++                      |                                          |
| R129   | T                      | T           | T               | 7 W           | —                              | ++                         | ++                       |                                          |
| R130   | T                      | T           | T               | 8 W           | —                              | +++                        | +                        |                                          |
| R131   | T                      | T           | T               | 6 W           | —                              | —                          | +                        | Much Wallerian degeneration and fibrosis |
| R133   | T                      | T           | T               | 8 W           | —                              | +++                        | ++                       |                                          |

T, Treatment given; O, treatment omitted; —, none; +, some; ++, many; +++, very many; W, week; d, days.

under a dissecting microscope. The teased mass was placed over the exposed surface of the spinal cord and tucked under the dura. In some cases it was also pushed into the substance of the cord in the region of the lysolecithin injections. The wound was closed and animals were killed at various times after these procedures (Table 1) by aortic perfusion with 4% glutaraldehyde in phosphate buffer under pentobarbitone anaesthesia. The cord was removed and 1–2-mm transverse slices from the injected area were post fixed in osmium tetroxide and embedded in Araldite. The effects of transplantation were assessed using 1- $\mu$ m sections stained with alkaline toluidine blue.

In all animals which received nerve transplants, with the

exception of cat R131, Schwann cell remyelination was present in the areas demyelinated by the injection of lysolecithin (Table 1) (Fig. 2). The extent of Schwann cell remyelination varied but in general was greater in the rats than the cats. Not all demyelinated axons were remyelinated, which in the rats may be related to the short survival times; however in the cats this failure to remyelinate completely may be due to other factors because the persistently demyelinated axons occurred deeper in the cord than those remyelinated by the Schwann cells. In the rats lesions in the ventral columns present in animals R109 and R110, showed no evidence of remyelination confirming the results found in the non-transplanted animals that the dose of irradiation used suppresses inherent remyelination.

These experiments show that it is possible to prepare small localised areas of primary demyelination in experimental animals which do not remyelinate (Fig. 1); in this they resemble the persistently demyelinated plaques of multiple sclerosis. When a source of Schwann cells is provided the axons are remyelinated by these cells (Fig. 2); this is most marked with axons close to the transplanted cells but more distant axons may remain demyelinated. Although the experimental system used to demonstrate this phenomenon is grossly abnormal, indeed is potentially lethal to the animal, it does show that it is possible to repair areas of demyelination which if left alone would remain demyelinated. The implication of this observation to human neurology must be considered, and factors which influence Schwann cell behaviour once they gain entry into the CNS should be studied. It remains to be determined if the presence of Schwann cells within the CNS is ultimately beneficial or harmful.

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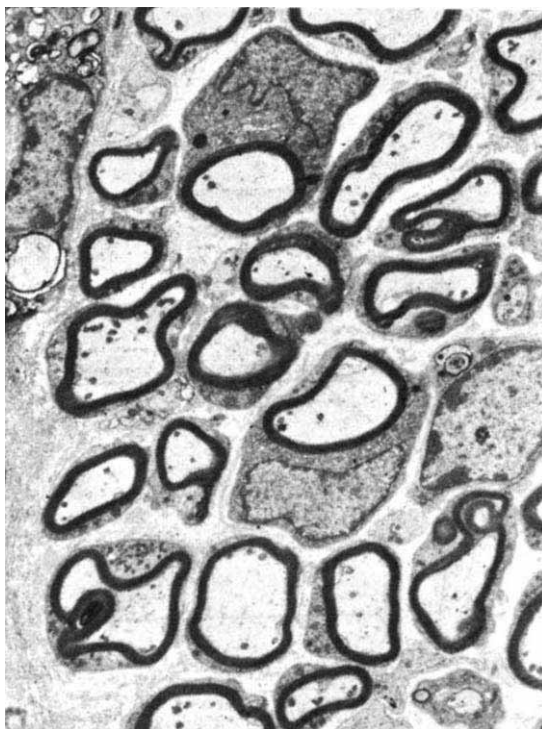


Fig. 2 Schwann cell remyelination following transplantation of sciatic nerve (rat R111).  $\times 4,400$ .

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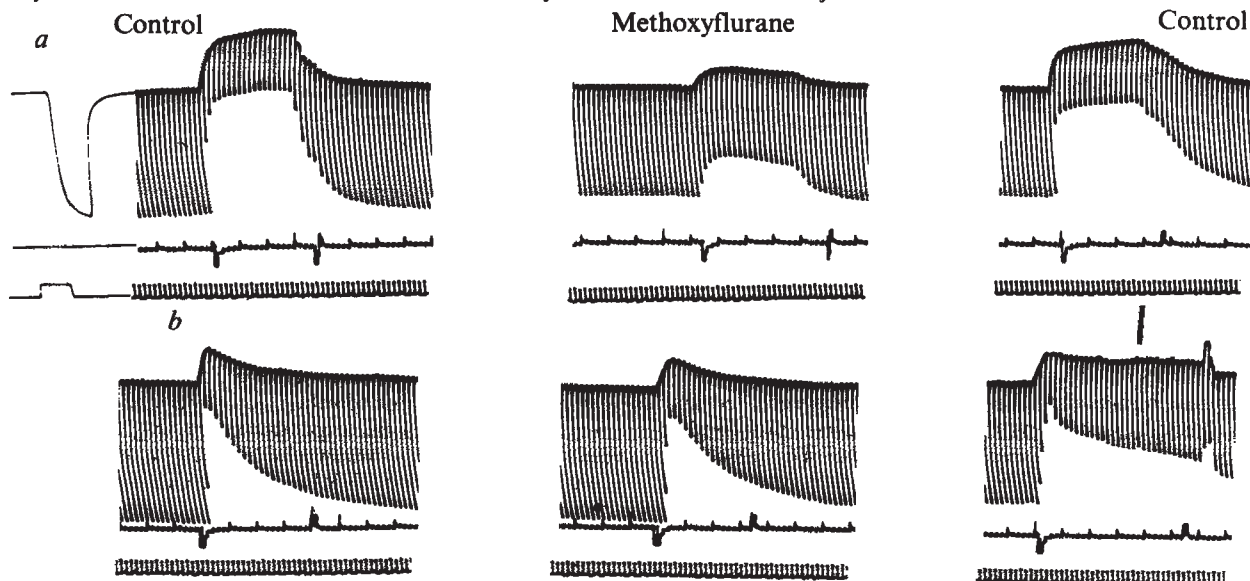


## Anaesthetic and convulsant ethers act on different sites at the crab neuromuscular junction *in vitro*

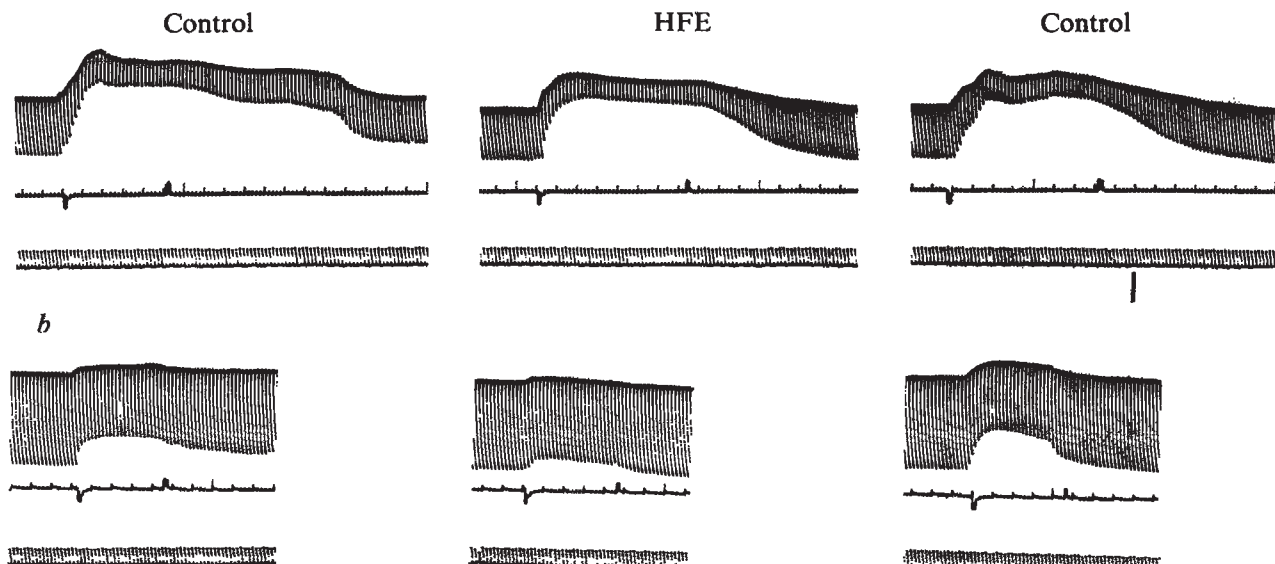
ALTHOUGH volatile general anaesthetics, of the so called structurally non-specific type, have been shown to affect synaptic transmission<sup>1,2</sup>, the underlying mechanisms are unknown. In view of their oil solubility, it has been suggested that these anaesthetics owe their depressant effect to the ability to induce non-specific disordering of membrane lipids<sup>3,4</sup>. But some equally oil-soluble and structurally non-specific ethers are potent convulsants rather than anaesthetics<sup>5</sup> and theoretical considerations suggest<sup>6</sup> that the two groups act at different sites in the nervous system and therefore are more specific in action than assumed so far. In support of this, we now report that each of a pair of representative agents, methoxyflurane, an anaesthetic and hexafluoroisobutylether

(HFE, Indoklon, Ohio Medical and Surgical Suppliers), a convulsant, have different effects on excitatory or inhibitory transmission at the crab neuromuscular junction. We have found that in the adductor muscle of the crab, *Ocypoda cursor*, which contains both glutamate and  $\gamma$ -amino-butyrate (GABA) receptors, methoxyflurane blocks the action of glutamate but not of GABA, while HFE blocks the effect of GABA but not of glutamate.

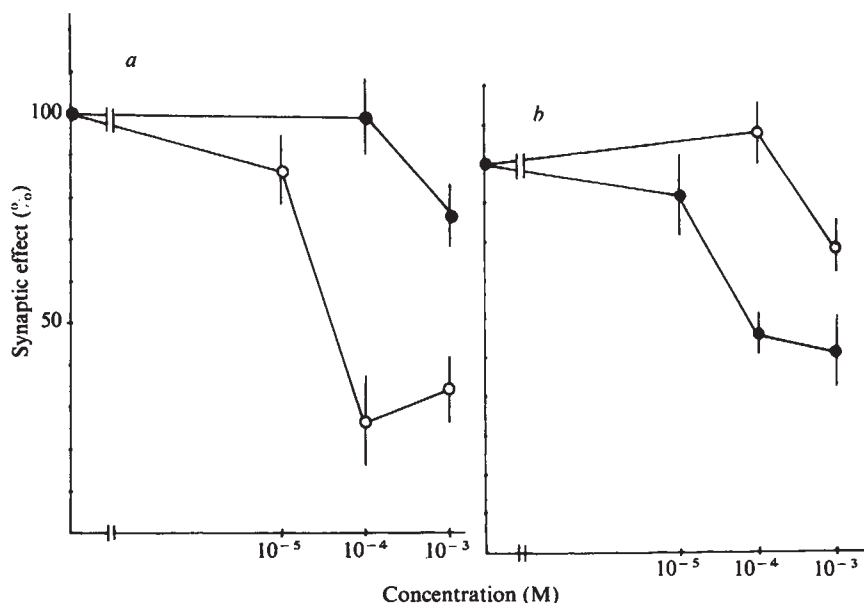
Muscle fibre cells were impaled with two micropipettes filled with 2 M KCl and having a resistance of 1–3 M $\Omega$ . One electrode recorded voltage and the other injected current. Usually, hyperpolarising current pulses of 100–500 nA and 100–200 ms were used to measure the effective input resistance of the cells. The voltage,  $V$ , and current,  $I$ , traces were amplified suitably and recorded on chart paper (Grass Polygraph model 79c). Membrane conductance,  $G$ , was computed as  $G = I/V$ . The preparation was perfused continuously with medium containing the desired drug at  $22 \pm 2^\circ \text{C}$ . The working concentrations of methoxyflurane and HFE were set at their clinically effective blood levels<sup>11,12</sup>.



**Fig. 1** Intracellular recording of the effect of  $10^{-4}$  M methoxyflurane,  $\text{CHCl}_2\text{CF}_2\text{OCH}_3$ , on glutamate-induced (*a*) and GABA-induced (*b*) conductance change in a cell from the adductor muscle of *Ocypoda cursor*, resting potential  $-75$  mV, input impedance  $60$  k $\Omega$ . *a*, Voltage changes caused by square current pulses of  $200$ -nA amplitude and  $100$ -ms duration in the following order: with  $5 \times 10^{-5}$  M glutamate; with glutamate and methoxyflurane; and with glutamate after removal of methoxyflurane. *b*, In the same sequence, voltage changes in the presence of  $5 \times 10^{-5}$  M GABA; GABA and methoxyflurane and GABA after removal of methoxyflurane. Time scale:  $1$  and  $5$ -s marks. Downward deflection on time scale indicates start of transmitter application; upward deflection indicates its termination. Calibration,  $5$  mV and  $500$  nA. In these and subsequent experiments, the composition of the bathing solution was, in mM: NaCl,  $453$ ; KCl,  $12.8$ ;  $\text{CaCl}_2$ ,  $34$ ;  $\text{MgCl}_2$ ,  $4.9$ ; Tris buffer,  $2$ . Muscle fibres were dissected and mounted according to Parnas *et al.*<sup>13</sup>.



**Fig. 2** Intracellular recording showing the effect of  $10^{-4}$  M HFE,  $\text{CF}_3\text{CH}_2\text{OCH}_2\text{CF}_3$ , on glutamate-induced (*a*) and GABA-induced (*b*) conductance change in a cell from the adductor muscle of *Ocypoda cursor*, resting potential  $-78$  mV and input impedance  $25$  k $\Omega$  (*a*) and  $45$  k $\Omega$  (*b*). (*a*) and (*b*). Voltage changes are shown in the same order and the deflections have the same significance as in Fig. 1. Calibration,  $5$  mV and  $300$  nA. Experimental conditions were the same for Fig. 1.



**Fig. 3** Dose dependence of the depressing effect of methoxyflurane (○) and HFE (●) on glutamate-induced (a) and GABA-induced (b) conductance change. The ordinate is percentage change with respect to control. Bars indicate standard deviation. Data for  $10^{-4}$  M are averages from eight cells; those for  $10^{-3}$  M and  $10^{-5}$  M are averages from the five cells each.

Figure 1 shows results from a typical experiment with  $10^{-4}$  M methoxyflurane. When at first the preparation was perfused with  $5 \times 10^{-5}$  M glutamate only, the muscle was depolarised and its input impedance decreased markedly. But after exposure of the cell to methoxyflurane for 5 min, followed by methoxyflurane and glutamate, the latter elicited a much reduced response. Eventually, when methoxyflurane was washed out, the original response to glutamate could be fully restored. This blocking effect of methoxyflurane was seen in eight cells where glutamate-induced conduction was reduced to  $26 \pm 4\%$  of control. In identical conditions, methoxyflurane did not block the action of GABA, which induced slight depolarisation and a marked reduction in membrane resistance. In eight experiments, the effect of GABA in the presence of methoxyflurane remained at  $108 \pm 3\%$  of control.

Opposite results were obtained with the convulsant, HFE (Fig. 2). No effect of  $10^{-4}$  M HFE was recorded on the action of glutamate. Thus, glutamate-induced conduction in the presence of HFE was  $99 \pm 3\%$  ( $n=8$ ) of control. On the other hand,  $10^{-4}$  M HFE blocked the synaptic effect of GABA, and GABA-induced conduction was reduced to  $56 \pm 2\%$  of control ( $n=8$ ). This apparent specificity of action in one type of synaptic transmission rather than in the other became less pronounced with higher concentrations of drugs. Thus, at  $10^{-3}$  M, either drug suppressed the action of both glutamate and GABA (Fig. 3): methoxyflurane remained more active with respect to glutamate while HFE was more active with respect to GABA.

We infer that the blocking mechanisms of these drugs at the molecular level are basically the same, but that they differ in potency with respect to function. Measurements in the exophase show that the equipotent molar ratio of methoxyflurane to HFE is about 1:100 in depressing glutamate transmission but 100:1 in depressing GABA transmission. Glutamate and GABA are believed to be excitatory and inhibitory transmitters in the crab, respectively<sup>7</sup>; and probably they have similar functions in the mammalian central nervous system (CNS)<sup>8,9</sup>. Extrapolated to CNS receptors<sup>2,10</sup>, our results may explain why methoxyflurane is an anaesthetic whereas HFE is a convulsant. Also, the specificity of action of each agent seems to be incompatible with a model whereby the drugs produce a non-specific disordering of bulk membrane lipids<sup>3,4</sup>. Rather, they seem to affect distinctive sites, probably functionally different proteins embedded in the same membrane.

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## Pathways for hydraulically and osmotically-induced water flows across epithelia

EVIDENCE linking an extracellular shunt pathway<sup>1</sup> for ions with intercellular junctions<sup>2</sup>, with reports that those junctions are permeable to large molecules such as sucrose<sup>3</sup>, inulin<sup>3</sup>, lanthanum hydroxide<sup>4</sup> and horseradish peroxidase<sup>5</sup> in various fluid-transporting epithelia suggests that water could also traverse this pathway. There is evidence, moreover, for a high hydraulic-conductance pathway across gastric mucosa<sup>6</sup> and rumen<sup>7</sup> if hydrostatic pressure is the driving force. Yet, except for the kidney proximal tubule<sup>8</sup>, measurements of osmotically-induced water flows across several 'leaky' epithelia have not in general yielded values<sup>9</sup> consistent with a high-conductance pathway. It has been argued, also, that the extracellular shunt is not a significant route for osmotic flow in the gallbladder<sup>10</sup>. To investigate these apparent discrepancies, we have measured the hydraulic ( $L_p$ ) and osmotic ( $P_{os}$ ) conductances of rabbit corneal endothelium. We report here that a large conductance for both hydraulic and osmotic flows either is present or can be demonstrated in appropriate conditions, and that its value is of the order expected for bulk water flow across the intercellular junctions.



The techniques for the dissection and mounting of the preparation, and the bathing medium's composition have been described before<sup>11</sup>. The corneal epithelium was scraped off. The rates of transendothelial fluid movement and the corresponding steady-state values of  $L_p$  and  $P_{os}$  were determined by an automatic technique<sup>12</sup> which maintains the volume of the outside compartment constant. The transient rates from which  $P_{os}$  at zero time was extrapolated were determined by microscopic measurements of corneal thickness<sup>13</sup>.

The effect of a hydrostatic pressure difference ( $\Delta P$ ) on the rate of fluid movement was determined as described in Fig. 1. The basal rate of fluid transport was usually measured with a  $\Delta P$  of 5 or 10 cm H<sub>2</sub>O. After each change in  $\Delta P$  there was a transient, followed by a period of reasonable stability. The effects were reversible during the 5–6 h of useful life of the preparation, then  $L_p$  increased progressively (Fig. 1), presumably due to cell deterioration. The long transients (Fig. 1) were attributed to changes in the thickness of the supporting stroma with  $\Delta P$ . Evidence came from separate observations of isolated stroma; endothelium was scraped off and silicone oil (20 cs) filled the inside (or aqueous) chamber, which limited water movements to the stromal-outer chamber interface. Transient changes in rate of water movement due to changes in  $\Delta P$  had the same amplitude and time course as those seen with endothelium present.

The results of all technically acceptable experiments are plotted in Fig. 2, and show that the flow is linear with the  $\Delta P$  for most of the range examined. At the low end of that range there is a trend towards an increased dependence of flow on  $\Delta P$ , but this point could not be substantiated by reversing the  $\Delta P$  because of technical limitations. For the linear interval,  $L_p$  was estimated

from Fig. 2 to be  $83 \pm 11 \mu\text{m s}^{-1}$  ( $V_w$  at  $37^\circ\text{C}$ :  $1.81 \text{ cm}^3 \text{ m}^{-1}$ ). 'Edge damage' was excluded as an influence in this value by mounting the preparation in the chamber, then clamping it with a cylindrical plug which left exposed only a 0.1-mm peripheral annulus. No leak was detected across this preparation even with a  $\Delta P$  of 150 cm H<sub>2</sub>O. To account for possible solute polarisation in the unstirred layers on both sides of the tissue, which could have led to an underestimation of  $L_p$  (ref. 14), we assumed that an increase in  $\Delta P$  induced an outward flow which diminished the apparent inward flow due to transport. Unstirred layer widths and NaCl diffusion coefficients were either taken from the literature or assumed (inside:  $\delta_1 = 350 \mu\text{m}$ ;  $D_1 = 1.54 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ ; outside:  $\delta_2 = 850 \mu\text{m}$ ;  $D_2 = 1.0 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ ); taking for the basal rate of fluid transport a value of  $J_v = 4.5 \mu\text{l h}^{-1} \text{ cm}^{-2}$  at a  $\Delta P$  of 10 cm H<sub>2</sub>O (Fig. 2), substituting into  $C = C_{\text{bulk}} \exp(-J_v \delta / 2D)$  (see later) for each interface and using  $\sigma_{\text{NaCl}} = 0.6$  (ref. 15) the corrected  $L_p$  is  $114 \mu\text{m s}^{-1}$ .

To determine  $P_{os}$ , inward flow was induced by making the inside solution hypertonic with sucrose, while outward flow was induced by making both sides initially 80 mOsm hypertonic with sucrose and then lowering the tonicity inside. The results summarised in Fig. 3 show that the steady-state osmotic flows are not linear with the osmotic pressure difference ( $\Delta\pi$ ), just as is the case in other epithelia<sup>9</sup>. These steady-state osmotic flows were, however, preceded by transients, which prompted an investigation of the osmotic flows obtained shortly after the imposition of a  $\Delta\pi$ . The results in Fig. 4 show that for the range  $10 < \Delta\pi < 80 \text{ mOsm}$  the initial rate of osmotic flow is linear with  $\Delta\pi$ . The slope for the linear region (Fig. 4) is  $166 \pm 12$

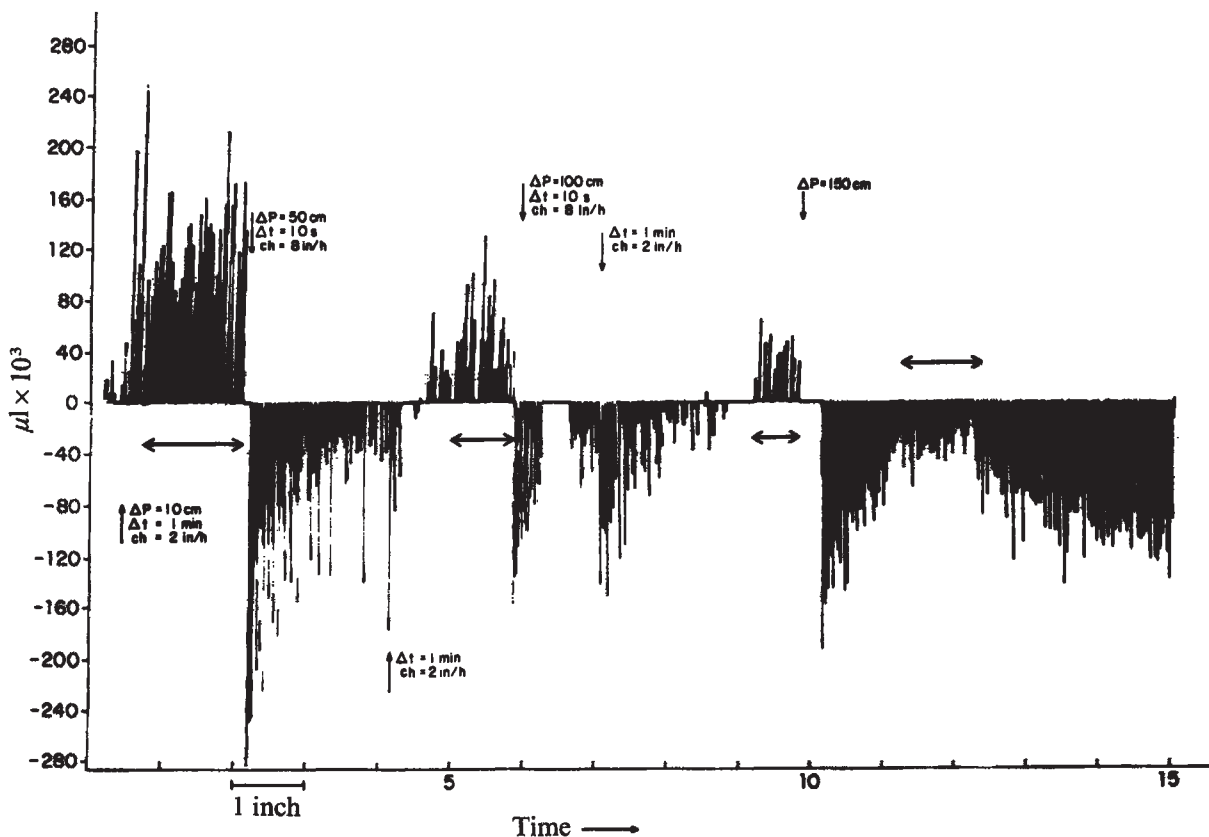


Fig. 1 Photograph of an experimental record. The height of the bars corresponds to the volume of fluid that traverses the preparation and is subsequently reinjected into (or aspirated from) the outside compartment by a stepping-motor-driven syringe. The recorder is reset to zero at time intervals  $\Delta t$ , so that a graphical display of the rate is obtained. The preparation was supported by a hemispherical stainless steel net, against which it was held by hydrostatic pressure applied on the inside (aqueous side). Chart markings are at 1-inch intervals; changes in  $\Delta P$ ,  $\Delta t$  and chart speed are shown at arrows. Positive ordinate denotes inward flow (from stroma to aqueous) and vice versa. In this experiment most of the stroma was dissected away. Average rates were computed from an accumulator for the periods denoted by horizontal bars.  $T = 36.80 \pm 0.01^\circ\text{C}$ ; area of preparation =  $175 \text{ mm}^2$ .

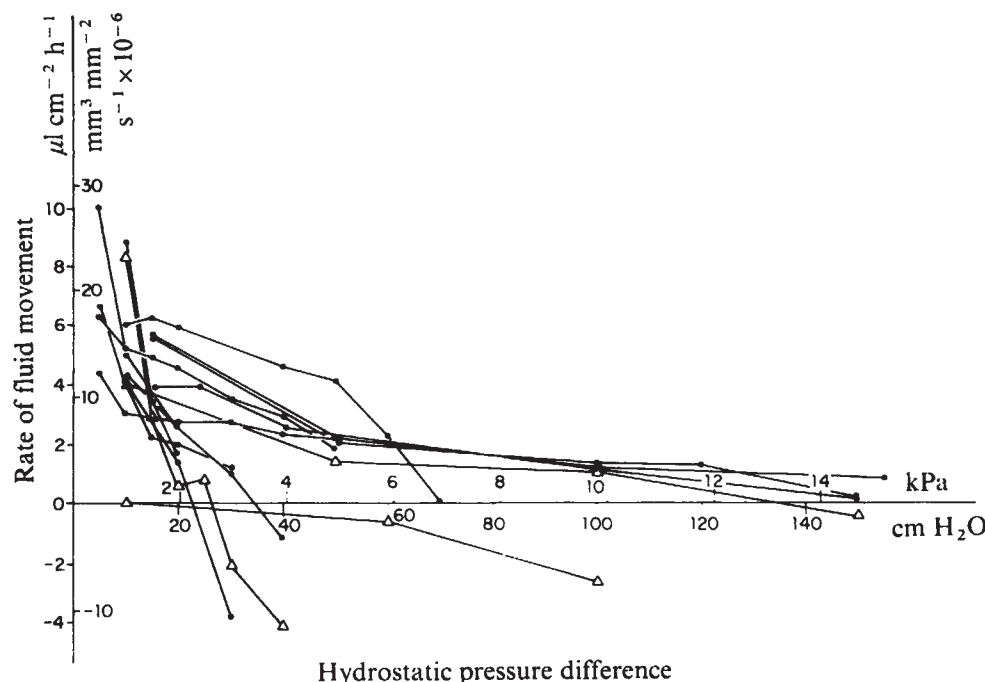


Fig. 2 Rate of transendothelial fluid movement with different values of hydrostatic pressure difference across that layer. Each curve represents an experiment; ●, the usual type; △, experiments in which most of the stroma was dissected away. Direction of flow and applied pressure as in Fig. 1. (kPa: kilopascals.)

$\mu\text{m s}^{-1}$ ; the chord is  $221 \pm 16 \mu\text{m s}^{-1}$ , which agrees with a value previously reported for the endothelial  $P_{os}$ . The error due to the delay imposed by diffusion of sucrose across the unstirred layer is estimated as 10–15% after the initial 15 s, so that for the present transients which last several minutes extrapolation appears permissible. A solution of the diffusion-convection equation  $\partial C/\partial t = D\partial^2 C/\partial x^2 - Jv\partial C/\partial x$  for the semi-infinite case is  $C(x,t) = C_0/2\{\text{erfc}[(x-Jv.t)/2\sqrt{(Dt)}] + \exp(Jv.x/D).\text{erfc}[(x+Jv.t)/2\sqrt{(Dt)}]\}$ , which for long times and comparatively small  $Jv$  tends to  $C = C_0 \exp(Jv.x/2D)$ . 'Natural convection'<sup>16</sup> in the boundary layer also runs counter to diffusional delays.

These results reveal either a high conductance ( $Lp = 114 \mu\text{m s}^{-1}$ ; steady-state  $P_{os} = 115 \mu\text{m s}^{-1}$ ;  $P_{os}(t=0) = 166$

$\mu\text{m s}^{-1}$ ) or a low conductance (steady-state  $P_{os} = 26 \mu\text{m s}^{-1}$ ), depending on the experimental conditions. The numerical values obtained for the high conductance are of a similar order of magnitude, suggesting that both hydraulic and osmotic flows could traverse the same high-conductance pathway. The dependence of steady-state  $P_{os}$  on  $\Delta\pi$  could be explained by progressive 'recruitment' of the high-conductance pathway at the higher values of  $\Delta\pi$ . We speculate that a similar mechanism might have a regulatory role in physiological conditions.

The possibility that the intercellular junctions may be the site of this high-conductance pathway is supported by arguments from two lines of reasoning. In the first, if the junctions are assumed to be water-filled slits which allow laminar flow, one has:  $Lp = a^3b/12\eta l$ , where  $a$  is junctional width,  $b$  is  $\frac{1}{2}$  total cell perimeter/unit area ( $10^3 \text{ cm cm}^{-2}$ ),  $\eta$  is viscosity of water ( $6 \times 10^{-3} \text{ P}$ ) and  $l$  is length of junctions (1  $\mu\text{m}$ ). For an  $Lp$  of  $114 \mu\text{m s}^{-1}$  ( $0.8 \times 10^{-11} \text{ cm}^2 \text{ s g}^{-1}$ ) the junctional width would be 39 Å. This figure is consistent with anatomical estimates (30–40 Å) and with the fact that horseradish peroxidase (estimated diameter: 45 Å) traverses these junctions<sup>5</sup>.

In the second, if the junctions are assumed to be impermeable to water, the osmotic pathway would consist of the basolateral and apical cell membrane in series. If they are assumed uniform, from the  $P_{os}(t=0)$  value, the  $P_{os}$  for the cell membranes would be  $200 \mu\text{m s}^{-1}$ . Although  $P_{os}$  values as high as this have been reported for erythrocytes<sup>17</sup>, in the present framework the value found for the low conductance would be difficult to explain. For instance, a collapse of the intercellular spaces which would exclude the lateral membranes from the osmotic pathway would reduce the endothelial  $P_{os}$  to some  $100 \mu\text{m s}^{-1}$ , rather than to the  $26 \mu\text{m s}^{-1}$  found. On the other hand, if the high conductance is attributed to the intercellular junctions, the transition to low conductance can be explained either by flow, osmolarity and time-dependent variations in the apparent reflection coefficient of the junctions for solute, or by an actual change in their conductance. In the extreme in which osmotic flow through the junctions becomes small, the low conductance value found would correspond almost entirely to a  $P_{os}$  of  $30 \mu\text{m s}^{-1}$  for the cell membranes. Such a value is of the order of those found for other animal cells<sup>9</sup> such as the "inner membrane" of frog skin epithelium<sup>18</sup> ( $24 \mu\text{m s}^{-1}$ ), and for some types of lipid bilayers ( $19 \mu\text{m s}^{-1}$ ) (ref. 19).

In summary, the possibility that the high-conductance pathway corresponds to the intercellular junctions whereas the low

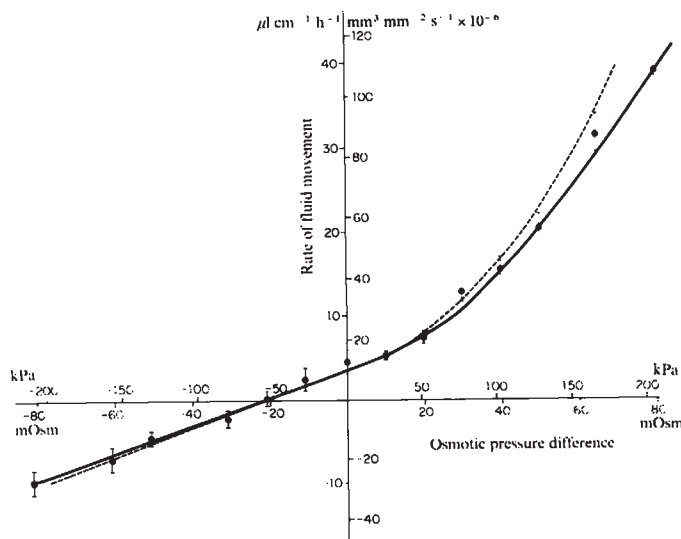


Fig. 3 Rate of transendothelial fluid movement induced by a difference in osmotic pressure. Average and s.e.m. values shown. The solid line was fitted by eye; the dashed line incorporates corrections for solute polarization (the values for the limiting slopes or apparent  $P_{os}$  are  $88 \pm 6$  and  $25 \pm 3 \mu\text{m s}^{-1}$  for the first and third quadrants, respectively; 'correction' changes these values to 115 and  $26 \mu\text{m s}^{-1}$ . Direction of fluid movements as above; positive osmotic pressure difference means aqueous hypertonic to stroma, and vice versa. The hydrostatic pressure difference was 10 cm  $\text{H}_2\text{O}$ .



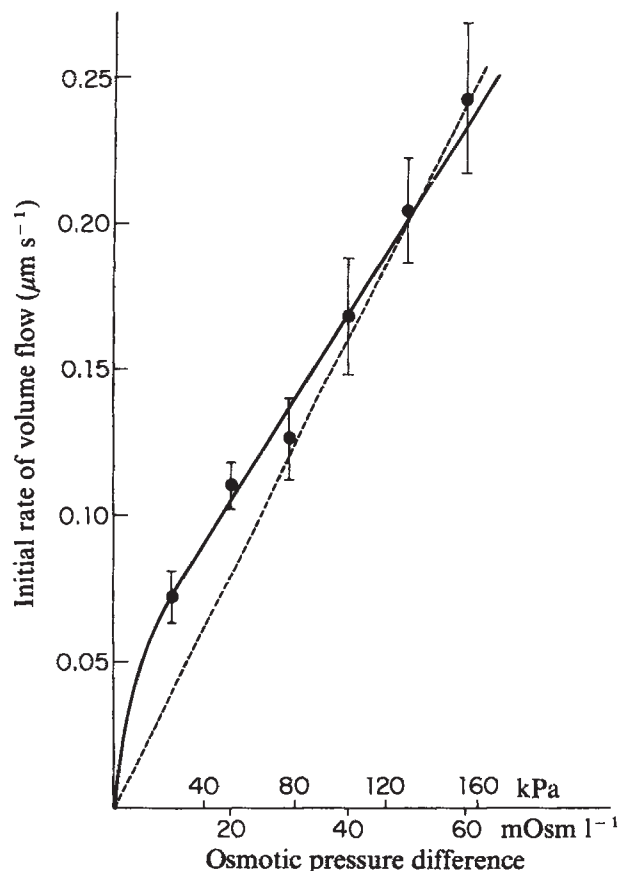


Fig. 4 Initial rates of osmotic flow (extrapolated at  $t = 0$ ) plotted against the driving force. Average and s.e.m. values shown. The solid line was fitted by eye; the dashed line represents the chord. The basal rate of fluid transport superimposed (approx.  $0.012 \mu\text{m s}^{-1}$ ) was neglected. The hydrostatic pressure difference was  $20 \text{ cm H}_2\text{O}$ .

conductance reflects properties of the cell membranes seems consistent with the present results. The detailed role of each pathway in the fluid transport mechanism across this and other epithelia remains a central question.

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## Experimental model of ulcerative colitis

AN experimental animal model of human ulcerative colitis has long been required, as there is no model with an immunological basis available. We report here the induction of inflammatory ulcerative changes in rat colon mucosa, similar to those of human ulcerative colitis. The lesions were consistently obtained by an experimental procedure that induces specific adult anti-foetal tissue immuno-reactivity.

Rat foetal colon implants beneath the renal capsule of syngeneic adult rats developed, by 6 d, acute inflammation and later ulceration of the mucosa morphologically indistinguishable from active ulcerative colitis. The sub-renal capsular implantation method for organ growth employed has been used as a model of delayed hypersensitivity of murine small intestinal mucosa in an allogeneic system<sup>1,2</sup>. In the present experiments, the syngeneic implantation permitted survival of the colon implants which, although not rejected, showed continuing immunological inflammatory damage of the mucosa. It is possible that ulcerative colitis and presumably other autoimmune diseases of man may follow or be exacerbated by immunoresponsiveness to foetal antigens, which may re-emerge in adult tissues in certain pathological conditions<sup>3</sup>.

The syngeneic rats were the Sprague-Dawley strain maintained in Monash University by brother-sister mating; they accepted tail skin syngeneic grafts but not allografts. Ten male (250 g) and ten female (190 g) rats received sub-renal-capsule implants of descending colon from 16-18-d-old foetal rats from two litters obtained by caesarean section of pregnant females of this inbred strain. Halves of each descending colon were implanted into male and female recipient pairs. A left lumbar approach to the left kidney of anaesthetised recipient rats was used in each case and the pairs of male and female recipients were killed at 48-h intervals, that is up to 20 d. The time scale of changes in the implanted colon was further investigated in five male and five female Sprague-Dawley rats which were killed in pairs 3, 5, 21, 28 and 32 d after implantation of colons from another litter of syngeneic foetal rats.

With the single exception of one rat after 28 d, the implanted syngeneic foetal colon remained structurally and functionally viable as shown by vascularisation by recipient blood vessels and continued production by the mucosa of intestine-specific mucin which stained positively by immunofluorescence with a specific anti-intestine serum and also

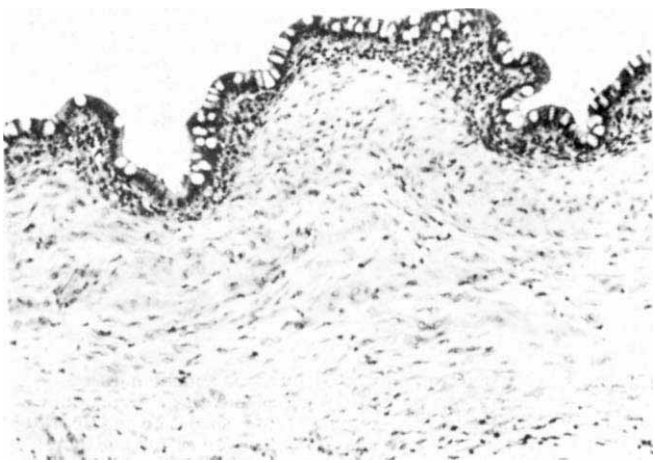


Fig. 1 Rat foetal colon implanted beneath renal capsule of syngeneic adult for 6 d. Note viable mucosa with prominent mucin droplets and a broad band of leukocytic infiltration beneath. (Paraffin section stained with haematoxylin and eosin;  $\times 90$ .)

with periodic acid-Schiff and alcian blue (pH 2.8). After 6 d the graft (in both male and female recipients) had become infiltrated by lymphocytes and eosinophils (Fig. 1). By 12 d many plasma cells were present and the mucosa was ulcerated with an abundant infiltration by neutrophil granulocytes at the sites of ulceration; the lumen of the graft also contained collections of granulocytes and large quantities of mucin (Fig. 2). Gradually over the succeeding days, the mucosa became depleted of mucin-secreting cells, and by 20 d it was largely destroyed. These changes were found consistently in all the pairs up to d 20. At d 28, the implanted foetal colon in one rat was reduced to a fibrous sac; in the other 28-d rat the implanted colon was well preserved overall, showing as the only pathological change, a microscopic mucosal diverticulum invested by an abundant focal lymphoid reaction. In one of the rats killed on d 32, there was again good preservation of the implant with only microscopic ulceration, in the other 32-d rat, the implant showed advanced changes such as occurred in the 12–21 d experiments.

The sex of the foetal rats donating implants was not determined in the 15 rats from three litters used in the above experiments, but a sample from each rat was

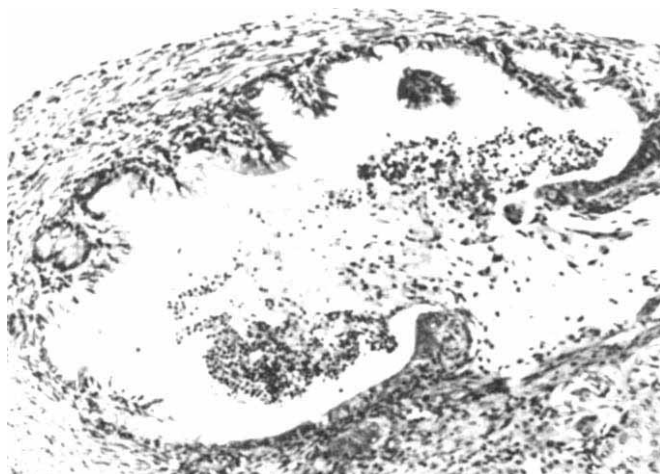


Fig. 2 Foetal colon implant at 12 d. Note ulcer lower right with conspicuous granulocytic exudate extending into lumen filled with mucin. Remaining mucosa looks viable but with less mucin production around the ulcer; it shows conspicuous submucosal leukocytic infiltration. (Paraffin section stained with haematoxylin and eosin;  $\times 90$ .)

implanted into male and female adult pairs. The findings in both male and female pairs of recipients were usually similar and no systematic differences were observed. We concluded that the pathological changes in the colonic implants are not attributable to sex-related antigenicity. To examine the strain specificity of the response, five male and five female Sprague-Dawley rats were given sub-renal-capsule implants of DA rat foetal colon. All of these allogeneic implants were totally destroyed within 10 d of implantation.

Serum samples from the majority of Sprague-Dawley rats 10 d after implanting syngeneic foetal colon, contained antibody reactive with foetal intestinal mucosa but not adult mucosa, as judged by indirect immunofluorescent staining<sup>7</sup> (Fig. 3). This indicates an immune reaction in the adult rats to foetal antigens deficient in the adult animal. Such a reaction has been demonstrated<sup>6</sup> by inoculating adult rats with syngeneic foetal intestinal material.

The lymphocyte and plasma cell infiltration and the serum antibody activity can be regarded as evidence of an immune response in the recipient to foetal antigens. The inflammatory and subsequent ulcerative reactions can also be attributable to immune rejection of foetal colonic

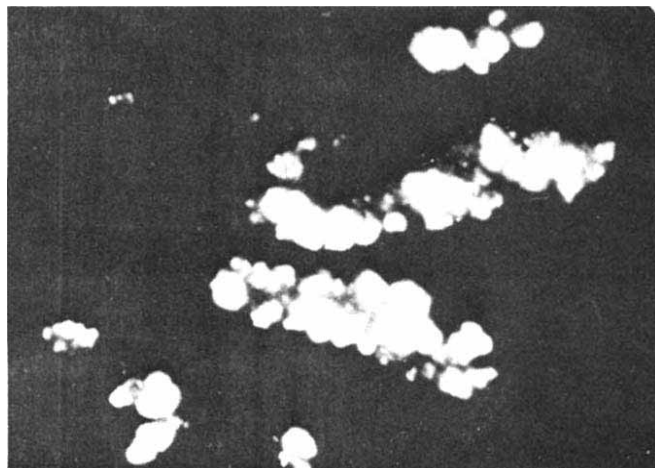


Fig. 3 Immunofluorescent staining of rat foetal colon mucosa by serum from adult rat implanted with foetal colon for 17 d. (Frozen section stained by sandwich immunofluorescence with fluorescein-labelled rabbit anti-rat globulin,  $\times 110$ .)

mucosa and experiments to establish this are in progress.

The morphological features of the lesions in the foetal colon implants are similar to those of human ulcerative colitis, which is also believed to have an immunological basis<sup>7</sup>. Patients with this disease have circulating lymphocytes which are cytotoxic to colon epithelial cells, and non-cytotoxic serum antibody directed against cytoplasmic components of colon epithelial cells. This rat system should provide a useful new model for investigating immunologically-determined colonic inflammatory disease. It will be interesting to see if it is possible to stimulate anti-colonic autoimmune activity in the recipient rats.

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## K-contractures and membrane potential in mammalian skeletal muscle

A FUNDAMENTAL property of skeletal muscle fibres is the ability to develop tension in response to surface membrane depolarisation. The relation between membrane potential ( $V_m$ ) and tension has been measured in amphibian muscle using external potassium concentration ( $K_0$ ) to clamp  $V_m$ <sup>1–4</sup>. A K-contracture follows exposure to high  $K_0$  and tension is graded with  $K_0$  between 20 mM ( $V_m = -50$  mV) and 50 mM ( $V_m = -40$  mV). Human intercostal muscle, however, either does not respond mechanically to  $K_0$  of 80 mM or gives a very small contracture<sup>5</sup>. It is not clear whether this is a result of diffusion delays in the large bundles of fibres or whether there is a basic species difference in excitation-contraction coupling. I report here that, in bundles of fewer than 20 fibres, the relationship between K-contracture tension and  $K_0$  is shifted to higher  $K_0$  in rat muscle because changes in  $K_0$  are less effective in altering  $V_m$ .



Adult male rats were killed by ether anaesthesia. The soleus, extensor digitorum longus (EDL) or sternomastoid muscle was isolated; bathed in solution A (Table 1), and bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub> during dissection at 20 °C. The sternomastoid has red and white segments and bundles of red or white fibres can be isolated<sup>6</sup>. Bundles were mounted in a rapid flow bath<sup>7</sup> at 37 °C. One tendon was fixed to an RCA valve and isometric tension recorded on a Grass Polygraph pen recorder. Twitch tension was 2.5 and 2.8 kg cm<sup>-2</sup> in two sternomastoid bundles (cross-sectional areas measured after fixation for electron microscopy). The tetanus to twitch ratio was between 4:1 and 5:1 in whole muscles and small bundles. Similar bundles from toad (*Bufo marinus*) semitendinosus muscles, in amphibian Ringers<sup>7</sup> at 20 °C, were also used.

Records from mammalian bundles exposed to 80 mM K<sub>0</sub> are shown in Fig. 1. Neither EDL (Fig. 1a) nor white sternomastoid (Fig. 1c) gave contractures although twitch tension fell quickly and recovered after the return to 3.5 mM K<sub>0</sub> within 30 min (EDL) to 1 h (white sternomastoid) without potentiation. Contractures from soleus (Fig. 1b) and red sternomastoid (Fig. 1d) bundles rarely exceeded twitch tension. Recovery of twitch tension in normal solution took 10 min (red sternomastoid) and 30 min (soleus) and was followed by potentiation. In the same system toad semitendinosus bundles gave contractures of four and nine times twitch tension with typical plateau and spontaneous decay phases. Recovery of twitch tension took

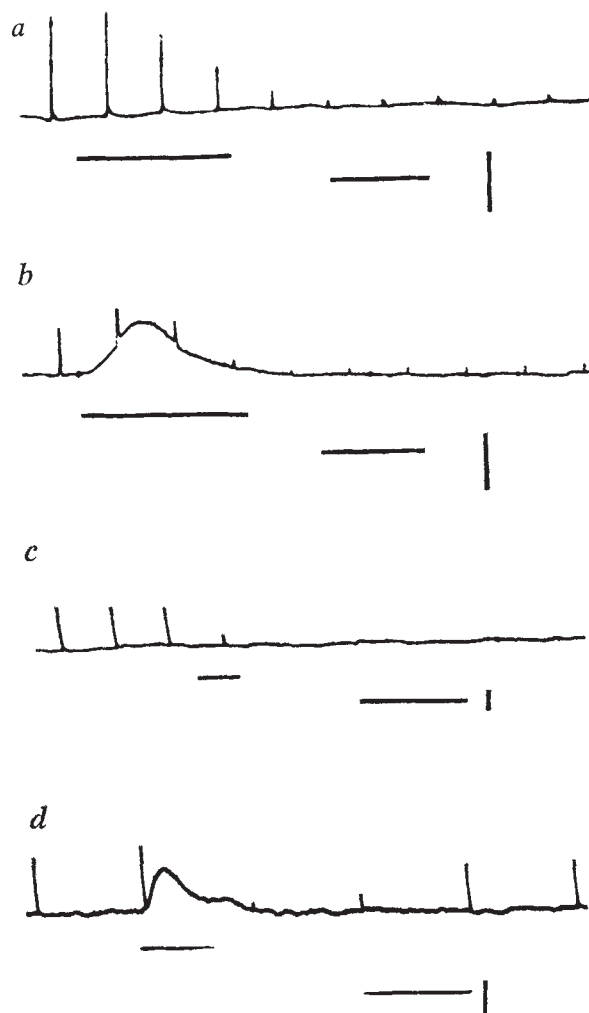


Fig. 1 Tension during exposure to 80 mM K<sub>0</sub> (Solution B, Table 1) for a period indicated by the horizontal bars under each record. a, EDL; b, soleus; c, white sternomastoid; d, red sternomastoid. Rapid vertical deflections are twitches (direct electrical stimulation). Scale: tension, vertical bar, 50 mg; time, horizontal bar, 20 s (a and b) and 60 s (c and d).

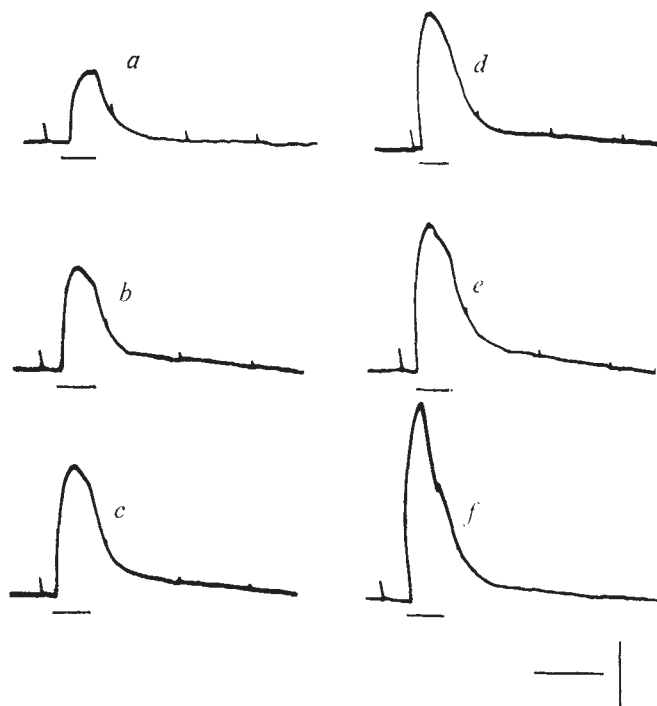


Fig. 2 Contractures from a bundle of red sternomastoid fibres exposed to different K<sub>0</sub> for a period indicated by the horizontal bars under each record. a 90 mM K<sub>0</sub>; b 112 mM K<sub>0</sub>; c 135 mM K<sub>0</sub>; d 158 mM K<sub>0</sub>; e 180 mM K<sub>0</sub>; f 200 mM K<sub>0</sub>. 45 min was allowed between contractures. Test solutions of K<sub>0</sub> up to 180 mM were made with appropriate proportions of solutions C and D (Table 1). Solution E was used for K<sub>0</sub> of 200 mM. The external chloride concentration was kept constant so that the K × Cl product varied. Scale: tension, vertical bar, 100 mg, time, horizontal bar, 60 s.

3 min in normal solution and was followed by potentiation. Clearly the responses of mammalian and amphibian bundles to 80 mM K<sub>0</sub> are significantly different, and rat muscles have a response similar to that reported for human intercostal muscle.

The responses (Fig. 1) seem to fall into two groups according to fibre type. The EDL contains mostly white fibres<sup>7,8</sup> and its response was similar to that of the white sternomastoid. The soleus, which contains mostly red fibres, responded in a manner similar to that of the red sternomastoid. Comparison of responses with twitch speed gave no consistent relationship since red and white sternomastoid bundles had similar contraction times and were five and ten times faster than the EDL and soleus respectively.

The smaller K-contracture in mammalian muscle was investigated further in red sternomastoid fibres by examining peak contracture tension and  $V_m$  when K<sub>0</sub> varied from 3.5 to 200 mM. The solutions were hypertonic for K<sub>0</sub> greater than 120 mM and so test and control solutions of 468 mOsm were used to avoid transient effects of tonicity on tension<sup>10,11</sup>. Twitch tension fell by 80% or more in the hypertonic control solution while tetanic tension fell by 10% so that the tetanus to twitch ratio was 20:1.

Table 1 Solutions used

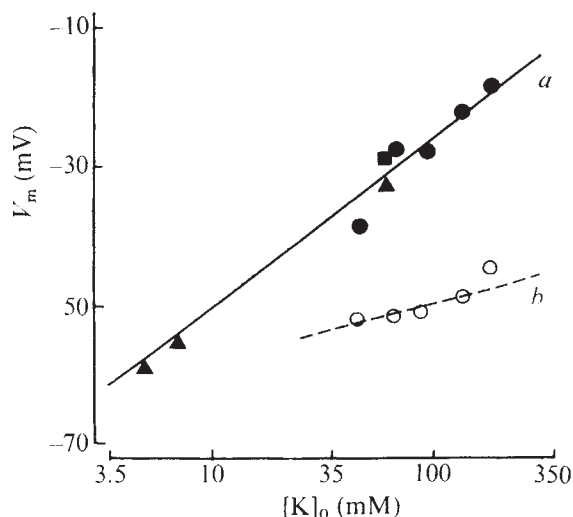
| Solution | Concentrations (mM)* |     |       |
|----------|----------------------|-----|-------|
|          | Na                   | K   | Cl    |
| A        | 165                  | 3.5 | 174.5 |
| B        | 88.5                 | 80  | 175.5 |
| C        | 206                  | 3.5 | 216.5 |
| D        | 28                   | 180 | 215.5 |
| E        | 28                   | 200 | 235   |
| F        | 28                   | 220 | 255   |

\*In addition each solution contained glucose 11 mM, Ca 2.5 mM, Mg 1.0 mM, HCO<sub>3</sub> 25 mM and HPO<sub>4</sub> 3.0 mM.

$K_0$  up to 200 mM induced no contracture in white sternomastoid bundles. Contracture tension in red sternomastoid bundles increased with  $K_0$  (Fig. 2). Tension in 200 mM  $K_0$  (Fig. 2f) was 10 times twitch tension but less than tetanic tension. Greater contracture tension might have been recorded if  $K_0$  above 200 mM had been used. Toad bundles developed maximum tension with 30–60 mM  $K_0$ . Higher concentrations (up to 120 mM) increased the rate of tension development, reduced plateau duration and increased the rate of spontaneous relaxation. Similar results have been reported for single frog fibres<sup>1,12,23</sup>. The kinetics of mammalian contractures (Fig. 2) showed a similar dependence on  $K_0$ . Tension in high  $K_0$  was shifted to higher  $K_0$  in mammalian muscle. This can be explained by a change either in the relation between tension and  $V_m$  or in the relation between  $V_m$  and  $K_0$ .

$V_m$  was recorded in red sternomastoid fibres using intracellular microelectrodes filled with 3 M KCl and the average resting  $V_m$  was  $-65 \pm 1.5$  mV. The steady-state  $V_m$  (after 3 min.) is plotted against  $\log K_0$  in Fig. 3: the line through the points has a slope of 28 mV, less than expected for a potassium electrode (61.5 mV) and less than reported for amphibian fibres (45 mV) with the same concentrations. The hypertonic conditions are not responsible for the observed slope, for points recorded in normal solutions fit the same line. The reduced slope would shift tension to higher  $K_0$  in mammalian muscle.

The time course of depolarisation of red sternomastoid fibres



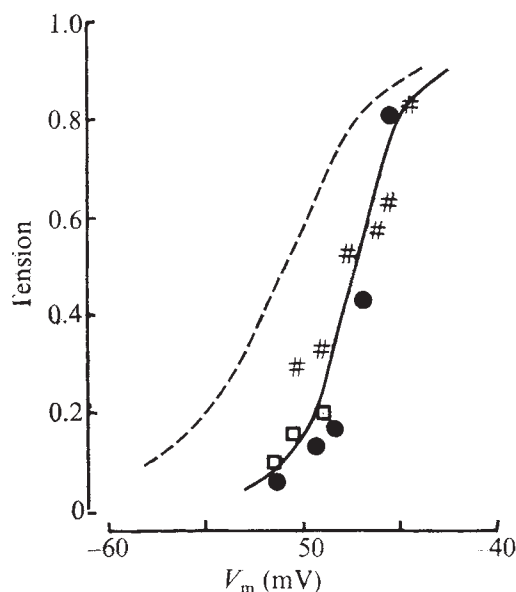
**Fig. 3**  $V_m$ , in mV, as a function of the logarithm of  $K_0$  in mM. ○,  $V_{m20}$ ; ●, steady-state  $V_m$  from the same fibres. Each point is the average of three or four fibres and contains the standard errors. Tetrodotoxin ( $2 \times 10^{-8}$  g ml<sup>-1</sup>) prevented action potentials and twitches. Bundles were stretched to 1.8 times rest length to reduce contractures. The fibres were equilibrated in hypertonic solutions for 1 h before the experiment. ■, fibres in isotonic solutions. Curve *a* was drawn through the points by eye and line *b* was drawn through the  $V_{m20}$  points assuming the relation between  $V_{m20}$  and  $\log(K_0)$  is linear.

in high  $K_0$  was surprisingly slow, taking 3 min, with a half time of 20–30 s, while repolarisation in 3.5  $K_0$  took 30 min. The time course was even longer in white sternomastoid fibres. But toad semitendinosus fibres, from bundles of a similar size, took 2 s to depolarise from  $-80$  to  $-60$  mV in 20 mM  $K_0$  with a half time of 4–5 s. It seems unlikely therefore that the time course of  $V_m$  in mammalian fibres is an artefact.

The relation between tension and  $K_0$  may be shifted to higher  $K_0$  by the reduced rate of change of  $V_m$  if depolarised mammalian fibres are subject to contractile inactivation in the same way as amphibian fibres<sup>1-3</sup>. Spontaneous contracture relaxation (Fig. 2) suggests inactivation, so that tension may be inhibited

before the steady-state  $V_m$  is achieved. Maximum tension was reached within 20 s (Fig. 2) and is therefore a function of  $V_m$  20 s after the solution change ( $V_{m20}$ ).  $V_{m20}$  is plotted against  $\log K_0$  in Figure 3 and is less than 50% of the steady-state  $V_m$ .

The reduction in K-contracture tension in mammalian muscle is a combined effect of the small change in the steady-state  $V_m$  and the slow time course of depolarisation following exposure to high  $K_0$ . In 80 mM  $K_0$  mammalian fibres respond to a  $V_{m20}$  of  $-51$  mV while amphibian fibres respond to a  $V_m$  that quickly approaches  $-30$  mV, a potential considerably more positive than the contracture threshold. The relation between tension and  $V_m$  in mammalian fibres is shown in Fig. 4. Comparison with data from ref. 1 (see Fig. 4) shows that the true relation between tension and  $V_m$  is similar in mammalian and amphibian skeletal muscle.



**Fig. 4** Tension (in relative units) as a function of  $V_m$  in mV.  $V_m$  was taken from  $V_{m20}$  (line *b* in Fig. 3). The symbols are peak tension during K-contractures and records taken from one bundle have the same symbol. The solid line was drawn by eye. The broken line is for amphibian skeletal muscle, ref. 1.

What are the reasons for the slope of 28 mV relating  $V_m$  to  $\log K_0$  and for the slow time course of  $V_m$  changes in sternomastoid fibres? The 28-mV slope (Fig. 3) indicates that another ion contributes to  $V_m$ . This is probably not sodium because the resting sodium permeability is low and ouabain ( $10^{-4}$  M) does not alter  $V_m$  in 60 min. The ion is probably chloride. Concentrations of chloride are not in equilibrium with the resting  $V_m$  in several preparations<sup>14-17</sup> and I have found that replacement of external chloride by sulphate increases the average resting  $V_m$  in sternomastoid fibres from  $-64$  mV to  $-76$  mV and increases the slope of the line relating  $V_m$  to  $\log K_0$  to 44 mV. The slow time course of  $V_m$  is unlikely to result from diffusion delays for reasons given above. 'Creep' arising from ion depletion in the T tubules<sup>18,19</sup> was recorded and is greater in mammalian fibres than in amphibian fibres with a time constant approximately 10 times longer. Restricted diffusion into the T tubules would increase the time course of changes in  $V_m$ .

Thus, bundles of mammalian fibres develop less tension in high  $K_0$  than bundles of amphibian fibres. The true relation between tension and  $V_m$  is similar in the two species but the relation between  $V_m$  and  $K_0$  for a given change in  $K_0$  is different in two respects; first the change in  $V_m$  for a change in  $K_0$  is 40% less in mammalian fibres and second the rate of change of  $V_m$  after an increase in  $K_0$  is more than 10 times slower.

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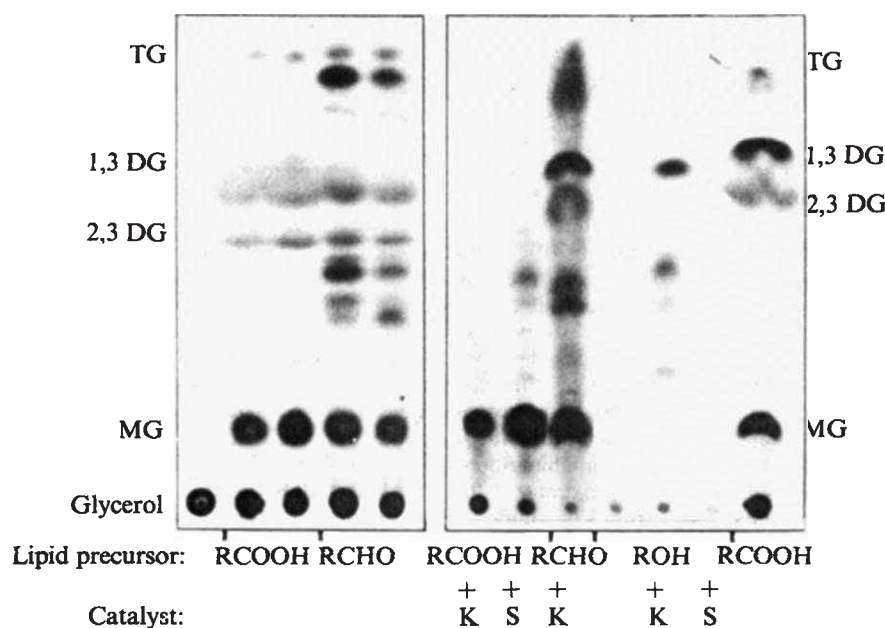
## Synthesis of phospholipids and membranes in prebiotic conditions

It is generally agreed that stable membranes were prerequisite to the assembly of the earliest self-replicating systems<sup>1-4</sup>. Phospholipids, which are ubiquitous in biological membranes and which self-assemble in aqueous environments into stable lipid bilayers and vesicles<sup>4</sup>, are obvious

candidates for prebiotic membrane components. We report here the abiotic synthesis of various lipids, including membranogenic phospholipids.

Our rationale in establishing simulated primaevial conditions included considerations of possible precursors, energy sources, catalysts and the behaviour and stability of the reactants and products. Derivatives of 1,2-diacylglycerophosphate are common membrane lipids, suggesting fatty acids, glycerol and phosphate as precursors. Heat derived from the Sun was a major primaevial source of energy<sup>5</sup>, and recent evidence indicates that average temperate zone surface temperatures may have reached 70 °C before  $3 \times 10^9$  yr ago<sup>6</sup>. The abiotic synthesis of polypeptides (molecular weight > 1,600) at 65 °C. in the absence of water or catalysts, has been detected in amino acid mixtures after incubation times of 40 d (ref. 7); we selected similar conditions for our experiments. Although various clays such as kaolin (an aluminum silicate) have been found to promote condensation reactions in aqueous conditions<sup>8,9</sup>, this mineral is rare in Precambrian sediments<sup>10</sup>. However, silicon oxide or silica is present in nearly all rocks<sup>11</sup>, and silicate rocks have been shown to promote condensations in certain conditions<sup>12</sup>. We tested the catalytic properties of silicic acid (silica gel H) and kaolin as possible models for natural silica-containing sands and clays. (Since completing this work, we have learned that washed and ignited sea sand promotes polypeptide formation when heated with mixtures of amino acid<sup>13</sup>.) Dicyanamide has been widely used as a possible prebiotic condensing agent, and as it has been shown to catalyse glycerol phosphorylation in acid solutions<sup>5</sup>, it was included in some experiments. Our general rationale was that tide pools containing dilute solutions of glycerol, phosphate and cyanamide, and having surface films of various hydrocarbon derivatives, could evaporate and produce mixtures of hydrophobic and hydrophilic compounds within a hot sand or clay matrix.

To synthesise neutral lipids, <sup>14</sup>C-glycerol and various C12 lipids were mixed in duplicate vessels and the solvent was



**Fig. 1** Autoradiograms of <sup>14</sup>C-glycerolipids including mono- (MG), di- (DG), and triglycerides (TG). 50 nmol <sup>14</sup>C-glycerol ( $2 \times 10^6$  d.p.m., ICN, Irvine) and 250 nmol C12 acid (RCOOH, Sigma), alcohol (ROH, Sigma), or aldehyde (RCHO, Aldrich) were placed in autoclaved microreaction vessels with loose fitting screw tops. In some cases, 10 mg kaolin (K, J. T. Baker, Phillipsburg) or silica gel H (S, Sigma), prewashed with acetone and chloroform-methanol, was added to the vessel before the removal of the organic solvent by vacuum. Reaction vessels containing aldehyde were sealed individually in small plastic canisters flushed with argon and containing a few CaCl<sub>2</sub> pellets. In one experiment using fatty acid and fatty alcohol, open test tubes were used, without desiccant (reactants were suspended in H<sub>2</sub>O which was evaporated during the first 2-3 d of incubation). Lipid yield from fatty acid was indistinguishable from the experiment using vacuum-evaporated microreaction vessels. After 1 week at 65 °C, total lipids were extracted in chloroform-methanol or ethyl ether and chromatographed on thin-layer plates of silica gel (F-254; EM Lab., Elmsford) twice in hexane-ethyl ether-acetate (100 : 200 : 1) (Fig. 1a), or once in a more complex solvent system<sup>20</sup> followed by the first solvent (Fig.

1b). Autoradiography was performed with Kodak RP-Royal X-omat film; exposure times of 3-7 d at -85 °C were followed by development in D19. One week exposure revealed 50-100 d.p.m. <sup>14</sup>C. Standard lipids were monolaurin (Sigma), dilaurin (Supelco, Bellefonte) and trilaurin (Sigma), run as internal standards in some samples and adjacent to unknowns in some cases. Identification of unknowns is tentative, as it is based solely on TLC R<sub>f</sub>s in several solvent systems (some samples were eluted and rechromatographed). Glycerol and lipids were used as received, except the aldehyde, which was purified on silicic acid in hexane and stored under argon.

removed by vacuum or by evaporation during subsequent incubation at 65 °C. Relatively short chain, saturated lipids were used as precursors for glycerolipids because they are fluid at 20–46 °C and are not labile to oxidation within the chain. In some cases, prewashed kaolin or silicic acid was added before the removal of the solvent. After drying, all samples were incubated for 1 week. Lipids were extracted and chromatographed on thin-layer plates of silica gel, placed over X-ray film for autoradiography, and the radioactive spots were scraped for scintillation counting. Typical autoradiographic patterns are shown in Fig. 1. Glycerol incubated alone showed only a single spot at the origin. Monoglyceride was the major reaction product from fatty acid, and was also prominent in the fatty aldehyde samples. Also tentatively identified were 1,3- and 1,2-diglycerides, and small amounts of triglycerides and several unknown lipids, possibly long chain cyclic acetals of glycerol. Total yields of glycerolipids were 3.1% from fatty acid and 7.7% from fatty aldehyde. Fatty alcohol formed little, if any, monoglyceride or other glycerolipid in the absence of added agents. With kaolin, however, detectable amounts of labelled lipids (0.2% yield) were formed.

Figure 2 compares the net yield of labelled lipid in the presence and absence of kaolin and silica gel. Surprisingly, kaolin seems to inhibit the formation of labelled lipids from fatty acid, but has much less effect on the reactivity of glycerol–fatty aldehyde. Silicic acid promotes the formation of monoglycerides from fatty acid (3.8% yield compared to 1.8% yield without catalyst), but inhibits the synthesis of more complex glycerolipids.

As initial experiments suggested that monoglycerides and more complex glycerolipids could have accumulated in evaporated tide pools, we selected an ether monoglyceride (chimyl alcohol) as a precursor for phospholipids. Ether monoglyceride is stable in the conditions used and avoided the requirement for large amounts of glycerol and fatty acid in the samples. Silicic acid, kaolin and dicyanamide were tested as possible condensing agents. Duplicate samples containing ether monoglyceride, dodecanoate and prewashed silicic acid and kaolin were mixed by vortexing in 2 ml H<sub>2</sub>O containing phosphate (20 mM) and glycerol (6.5 mM), or sodium dicyanamide (50 mM), or both (Table 1). All tubes were incubated sealed for 12 h at 65 °C, then all but one pair were allowed to evaporate to dryness in the vented oven. After 2 d the open tubes were completely dry in appearance and, after another 7 d, all tubes were extracted with chloroform–methanol–0.1 M KCl (ref. 14). Polar lipids were precipitated from acetone and chromatographed on thin-layer plates.

The polar lipid fraction from sample 2 (Table 1) contained phospholipids with *R<sub>s</sub>* similar to phosphatidic acid

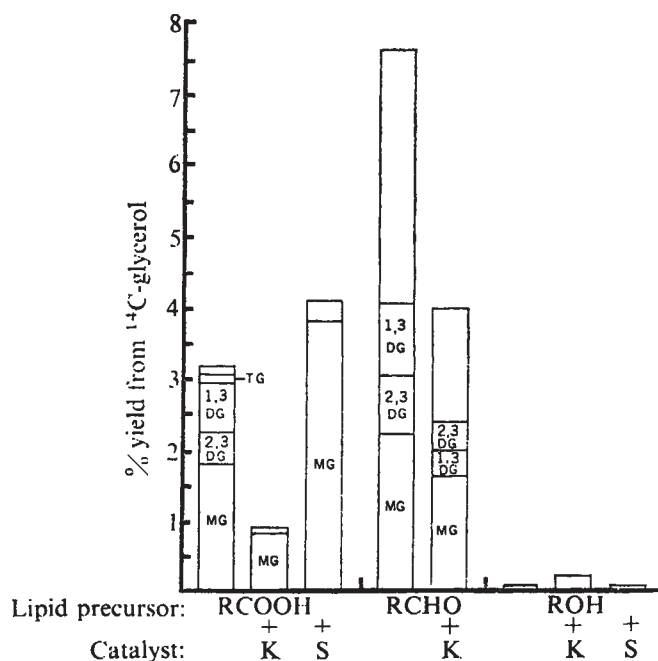


Fig. 2 Quantitation of <sup>14</sup>C-glycerolipids from C12 acid (RCOOH), aldehyde (RCHO) and alcohol (ROH). Radioactive lipids detected by autoradiography were scraped from TLC plates into scintillation vials. One ml H<sub>2</sub>O was added and after 1 h, 5 ml cocktail ('Tritonol' modified from Fricke<sup>21</sup>) was added. Percentage yield is based on estimated initial  $1.8 \times 10^6$  c.p.m., though total c.p.m. recovered from samples free of K and S were substantially lower, due to vaporisation of glycerol and adsorption to containment canister when present. RCHO samples contained substantial amounts of apparent triglycerides, but it was not feasible to count these spots separately from large amounts of an unknown lipid running just below. Unlabelled sections of bars in all other cases represent unidentified compounds.

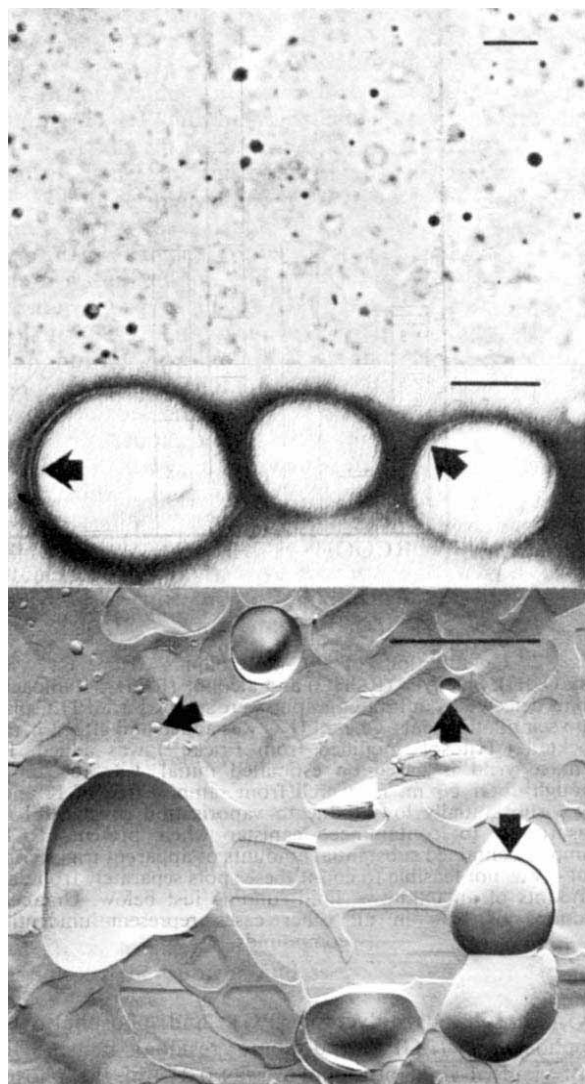
(PA), phosphatidyl glycerol (PG), and phosphatidyl glycerophosphate (PGP), and some residual neutral lipids. Total yield of lipid phosphorus was 0.2% of the phosphate in the reaction mixture. Samples 1 and 4 each yielded <0.015% lipid phosphorus, suggesting that dicyanamide and evaporation together enhanced phospholipid synthesis more than tenfold. Several polar lipid-like compounds (ninhydrin negative) were present in sample 3, but no phospholipids were found.

To determine if the partially purified phospholipids could form membranes, an aliquot from the polar fraction of

Table 1 Synthesis of phospholipid. All samples contained ether monoglyceride (chimyl alcohol, 10 mg, Sigma), dodecanoate (10 mg), prewashed K (50 mg), prewashed S (50 mg), and sodium azide (0.01% initial concentration). When present, phosphate was 20 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.8, glycerol was 6.5 mM, and sodium dicyanamide (Aldrich) was 50 mM. Lipids extracted from alkaline samples after addition of 0.1 M KCl (ref. 14) were only partly soluble in chloroform (C) or ethylether (E), but readily soluble in C–methanol (M) (2 : 1). Polar lipids were precipitated by adding 10 volumes of acetone to 1 volume of C–E (1 : 1) containing partially solubilised lipid and storing at –20 °C overnight. Insoluble (polar) lipids were collected after centrifugation in a clinical centrifuge at 4 °C. Phospholipid was identified by spray reagent<sup>22</sup> and by the method of Lowry *et al.*<sup>23</sup> after scraping spots from charred preparative plates of silica gel H run in C–M–H<sub>2</sub>O (65 : 25 : 4). Compounds similar to PA, PGP and PG were present at greater than 0.065, 0.016, and 0.005 μmol lipid phosphate in the acetone fractions of sample 2. Samples 1 and 4 each contained less than 0.006 μmol total lipid phosphorus.

| Sample | Treatment             | Phosphate | Glycerol | Dicyanamide | Phospholipid detected |
|--------|-----------------------|-----------|----------|-------------|-----------------------|
| 1      | Aqueous               | +         | +        | +           | Trace                 |
| 2      | Evaporated to dryness | +         | +        | +           | 0.2% Yield            |
| 3      | Evaporated to dryness | –         | –        | +           | None                  |
| 4      | Evaporated to dryness | +         | +        | –           | Trace                 |





**Fig. 3** Vesicles from polar lipid extracts of sample 2. *a*, Phase contrast micrograph (bar is 10  $\mu\text{m}$ ) of lipid suspension ( $1 \text{ mg ml}^{-1}$ , made by evaporating solvent from aliquot in small tube, adding 30 mM NaCl, 2 mM  $\text{Na}_2\text{HPO}_4$  (pH 7.4), and warming to 50–55  $^\circ\text{C}$  for 1 min. *b*, Negative stain electron micrograph (bar is 0.1  $\mu\text{m}$ ) of above preparation after probe sonication (Bronwill Biosonik, Will Corp., Rochester, New York) for 10 s at 53  $^\circ\text{C}$ . Vesicles were stained with uranyl acetate (1%) after sample was applied to carbon-formvar coated grid and blotted dry. Note several lamellae (arrows). *c*, Freeze-fracture electron micrograph (bar is 1.0  $\mu\text{m}$ ) of lipid suspension ( $5 \text{ mg ml}^{-1}$ ) made by warming lipid film as above with the buffer containing 25% glycerol as a cryoprotectant. Note vesicles of various sizes (arrows).

sample 2 was dried in a small tube. The sample was suspended in 30 mM NaCl, 2 mM  $\text{Na}_2\text{HPO}_4$  (pH 7.4) by warming to 50  $^\circ\text{C}$  in a water bath. After a few seconds, a slightly opalescent solution with no visible particles was obtained. Phase contrast microscopy revealed a heterogeneous suspension of apparent vesicles (Fig. 3*a*). Brief sonication with a probe sonicator at 50–55  $^\circ\text{C}$  resulted in a more homogeneous population of vesicles (negative stain electron micrograph, Fig. 3*b*). A concentrated suspension, made without sonication in the above buffer containing 25% glycerol, was prepared for freeze-fracture electron microscopy. This technique exposes the hydrophobic plane of lipid bilayer membranes and preserves the structure of lipid-water phases<sup>15</sup>. The presence of lipid vesicles of various sizes in this preparation (Fig. 3*c*) confirmed our

light microscope and negative stain electron microscope observations.

This work has implications concerning the possible origin of life on Earth. Although considerable progress has been made in the abiotic synthesis of important biomolecules, including phospholipid precursors, in simulated primaeval conditions<sup>6,16</sup>, the only similar studies of prebiotic glycerolipid synthesis are those of Degans *et al.*<sup>8,9</sup>, who reported the synthesis of neutral lipids from glycerol and fatty acids in a water-kaolin mixture held at 85  $^\circ\text{C}$  for 2 weeks. Neither reagent purity nor control experiments were described, however, and yields were relatively small (about 0.1%). Our results show that both fatty acid and fatty aldehyde react rapidly with glycerol in the absence of catalysts to form the precursors of membrane lipids, and that phospholipids and lipid-membrane vesicles can assemble in possible prebiotic conditions. We suggest that silicates other than kaolin are likely to have promoted such syntheses.

It is significant that phosphatidyl glycerol was apparently formed in our reaction mixture. This lipid, and its aminoacyl derivatives, are prominent in most prokaryotic membranes<sup>17</sup>. Our results show that membrane-forming lipids require only three precursors: glycerol, fatty acid or aldehyde, and phosphate. An alternative and well-studied model system for prebiotic membranes consists of proteinoid 'microspheres' prepared from concentrated solutions of amino acid polymers<sup>18</sup>. There is general agreement, however, that biological membranes require lipid bilayers as barriers to free diffusion of metal ions and substrate molecules. Because our results show that membranogenic lipids are readily produced in simulated prebiotic conditions, we concur with suggestions (Goldacre in ref. 4)<sup>3,4,19</sup> that lipid bilayer membranes could have been present before the accumulation of proteins, polynucleotides and other complex molecules.

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# Student books supplement

## Recommended reading

*During the past few years several publishers have produced series of books or pamphlets on specialist topics designed for use on undergraduate courses, particularly those in biological disciplines. Seven reviewers with experience of teaching such courses present here their impressions of the extent to which these series have been successful, in their particular fields, as additional teaching material for students.*

THERE was a time when individual authors were able to write encyclopaedic works about their subject, and they frequently conveyed their information with an air of bland authority. In botany, names such as Sachs, Strasburger, Kerner and Oliver will generate waves of nostalgia among the older generation of plant scientists. In the early decades of this century, undergraduate courses could be taught using just one or two major texts as background reading. Modern single-author textbooks are usually far more restricted in the topics they cover and rarely claim to summarise all knowledge concerning that subject; more often they are content to illustrate the basic principles of their subject by reference to specific examples.

There have been some attempts at the encyclopaedic approach even in recent years, but such efforts have not been greeted with enthusiasm by the academic world. In these days of intense specialisation the experts in each of the topics covered are sure to find fault with the section dealing with their pet topic in any such book. And, since no student will read a textbook from cover to cover, if they cannot have certain sections specifically recommended, they are unlikely to pick up the book at all.

Multi-author books, sometimes with one or two editors involved in their production, represent one answer to the publisher's dilemma. Such books are often very successful, but may suffer from an uneven style and quality. An alternative is the series of monographs, each written by a specialist in the field, and each relatively independent of the others. This overcomes inconsistencies in style and permits a relatively low cost for each volume. This approach has been taken by many publishers both at research and at undergraduate levels. Pioneers in the undergraduate biology sphere have been Edward

Arnold (London), publishers of the *Studies in Biology* series (hardback and paperback editions).

Overall, this series has probably been more successful than any other which has been attempted. The secret of its success lies in the publisher's skill in the selection of topics and determination of length (and, thereby, cost). For example, the first issue of the series was *Ecological Energetics* by John Phillipson (£2.25; 95 pence) and this has become a classic of ecological literature within its ten years of life. The topic is sufficiently well defined to be outlined in about fifty pages and has been admirably condensed by the author. Similarly successful issues have been *Plants and Water* by J. Sutcliffe (£1.30 paperback), *Population Dynamics* by M. E. Solomon (£2.20; £1.10), and *Photosynthesis* by D. O. Hall and K. K. Rao (second edition, March 1976; £2.80; £1.40). It is difficult to avoid the temptation to produce broad topic books—such as *Biology of Pollution* by Kenneth Mellanby (£1.75; 75 pence) and *Microecology* by J. L. Cloudsley-Thompson (paperback £1.20)—which may do well on the bookstalls because of their general appeal, but which try to cover too great a scope to be valuable as course texts. The opposite end of the spectrum is also to be found in the series, particularly in the later issues (are the publishers running short of ideas or authors?). This extreme is represented by topics which are concise to the point of having limited appeal, such as *The Biology of Eucalypts* by L. D. Pryor (£3.20; £1.60) and *The Secretion of Milk* by Ben Mephram (£2.80; £1.40).

*Plant Taxonomy* by V. H. Heywood (£2.60; £1.30) has now entered a second edition (1976) and forms a useful introduction to experimental taxonomy. It contains some interesting chapters on

chemical and numerical approaches to the subject which help to dispel the old-fashioned image of this subject. *Genetics and Adaptation* by E. B. Ford (£2.60; £1.30) is a new issue which is bound to sell well. The basic genetics are dealt with in two short chapters and the remainder of the book has a distinctly ecological flavour, dealing with such subjects as selection, melanism and isolation. This should be widely recommended as a background to a variety of biological courses; all first-year biology students should read it.

Tissue culture is now a frequently used technique with which most advanced level school biologists will have had some contact. *Plant Tissue Culture* by D. N. Butcher and D. S. Ingram (£3; £1.50) provides a guide to the implications and applications of the technique and also supplies the practical information needed for setting up simple tissue culture experiments in the laboratory.

Two more specialised studies which have been issued during the past year are *Phytochrome and Plant Growth* by R. E. Kendrick and B. Frankland (£3; £1.50) and *Lichens as Pollution Monitors* by D. L. Hawksworth and F. Rose (£2.80; £1.40). The former is a timely review of a fascinating and fast developing area of plant physiology, and once again it includes simple laboratory experiments. The second book is really an expansion of the authors' paper in *Nature* (227, 145; 1970). It makes interesting reading, but is rather specialised for this series and suffers from the disadvantage that the taxonomy of lichens is sufficiently difficult to put the application of this technique for pollution detection out of the reach of the average undergraduate. Species descriptions are included but a simple, illustrated key to the more important indicator species would have made this a much more useful book.



The *Tertiary Level Biology* series (hardback and paperback), published by Blackie (Glasgow and London), also aims at the undergraduate, but has a more advanced approach, thus making it of value to postgraduates too. *Water and Plants* by H. Meidner and D. W. Sheriff (£5.50; £2.90) is the latest issue in this series, and begins by describing the properties of water and the concept of water potential. In a logical order it then considers water vapour and transpiration, water transport in the plant, and the plant-soil relationship. This is certainly not an introductory book and the undergraduate would need to be weaned on a lower level text, such as the Edward Arnold study by J. Sutcliffe. It does, however, provide a full, thermodynamic and mathematical basis for understanding the current state of the study of plant-water relations.

Hutchinson (London) have also begun a series of introductory studies in biology. The third and latest title in this series is *Microbial Plant Pathology* by P. J. Whitney (£4.95; £2.95). This is a well-produced and readable book which assumes no previous knowledge of the subject. It begins with an account of disease transmission which concentrates on crop diseases, and which underlines the economic importance of plant pathology. It then considers infection, defence mechanisms and pathogen-induced reaction in the host species. It is a clear and concise introduction to the subject and is good value for its price.

One of the latest issues in the *Oxford Biology Readers* series (Oxford University: London) also deals with plant pathology and is entitled *The Control of Plant Disease* (30 pence), by B. E. J. Wheeler (who, incidentally, is also author of *Diseases in Crops* in the Edward Arnold series). The idea behind the *Oxford Biology Readers* is brevity and, consequently, low price. These aims are undeniably achieved, but at the expense of depth. Usually they are about 15–30 pages long, and this kind of treatment demands very careful selection of material. Some subjects are more suitable for a brief survey than others. Plant pathology cannot be covered adequately in 15 pages although Wheeler does achieve an interesting introduction to the problem of disease control. The topic chosen for an issue in this series must, therefore, be relatively narrow in scope. For example, in the issue entitled *Stomata* (60 pence), Heath admirably attains a detailed analysis of the physiology and mode of action of these organs.

*Oxford Biology Readers* are performing a very different function from the other monographs reviewed here in that they have such limited coverage

within each issue. Because of this they do not generally form a basis for a specific undergraduate course but provide supplementary information on certain topics within such a course. This must limit their sales potential among undergraduates despite the high quality of some of these booklets.

Having examined several of the biological series currently being served up for undergraduate consumption, one cannot help being impressed by the Edward Arnold's *Studies in Biology*. These appear to have struck the best length of treatment and selection of topics to meet the widest student demand. The publishers should be congratulated on anticipating so accurately the growing demand for this type of publication.

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BIOLOGY has become such a diverse subject, and one in which there are so many rapidly-developing areas, that the single, comprehensive textbook covering the whole field has become largely obsolete. A new development has been the production of series of smaller, cheaper books, to which new, up-to-date titles on specific topics can be added as they become appropriate. Such series have the advantage that lecturers and teachers can selectively stock their libraries with material that is directly relevant to their particular courses without having to pay for additional material they do not require.

The *Oxford Biology Readers* series (Oxford University: London) are essentially a series of essays on a variety of biological topics—some very specific, others more general—written by authors who are pre-eminent in their particular fields. Each provides a concise and authoritative introduction to its subject. They are well produced with clear photographs and excellent diagrams and they include fairly comprehensive suggestions for further reading. They are excellent value and provide an invaluable source of basic information for students at advanced school and undergraduate levels.

In *Primate Locomotion* (45 pence) Professor J. R. Napier describes the variety of locomotor patterns found in primates and discusses how these have evolved in relation to the habitats in which primates live and the various types of food that they eat. He also discusses the rather scanty evidence relating to the locomotion of fossil primates and the special skeletal adaptations for bipedalism shown by man.

*Insect Flight* (45 pence) by Professor J. W. S. Pringle deals with many aspects of a complex subject in a remarkably clear and concise way. He discusses the

different mechanisms found in insects, the aerodynamics of flight, the physiology of insect flight muscles and the control and coordination of flying. This *Reader*, which is illustrated by exceptionally clear diagrams, can only provide a rather cursory examination of such topics, but perhaps its principal value is that it encourages the reader to pursue the subject further.

The Echinoderms are a group of animals that differ from other Phyla in so many ways that there is a tendency to regard them as somehow being off the main stream of evolution. Professor D. Nichols, in *The Uniqueness of Echinoderms* (45 pence) sets out to convince the reader not only that they have a fascination in their own right but that (because of their abundance in the marine environment and the fact that, through the echinoderm larva, they may be the ancestors of the chordates) they are entitled to more attention from zoologists. His enthusiasm for these animals is evident in this lively and interesting little book.

In *Evolution Studied by Observation and Experiment* (45 pence), Professor E. B. Ford discusses the contribution to our understanding of evolution made by the study of ecological genetics, a field in which he has been a major influence. He shows how the study of various characters under natural conditions has revealed that selection pressures favouring advantageous characters are much more powerful than Darwin and other early evolutionary theorists had believed was possible and that natural selection can bring about adaptive changes very much more quickly than was previously supposed. His evidence is drawn from studies by himself and others, including those on colour patterns in butterflies, shell colouration in snails and the human blood groups.

During the development of the science of animal behaviour certain theoretical concepts have been developed and have subsequently been the focus of considerable debate and disagreement. Robert and Joan Hinde discuss two such concepts in *Instinct and Intelligence* (45 pence). Using a wealth of examples they show how instinct has become a somewhat disreputable concept, whereas intelligence now has a precise scientific meaning, more restricted than its everyday usage. They argue strongly against using either concept as an explanation for behavioural phenomena and suggest that they are important as ideas that point to problems and to areas of investigation, which should ultimately make them redundant.

The story of the evolution of the birds from the reptiles through the fossil *Archaeopteryx*, has been told many times. In *The Evolution of Flying and Flightless Birds* (45 pence), Sir Gavin de Beer provides a concise and well-illustrated

discussion of this topic, together with the evolution of the ratites, a group which, because of their flightlessness and geographical distribution, have exercised the minds of evolutionary theorists for many years. Since de Beer's death in 1972 there have been some interesting developments in this field, notably Ostrom's re-analysis of *Archaeopteryx's* wing and Cracraft's theory that the ratites are descended from a single flightless ancestor which was dispersed by continental drift.

All these *Readers* can be highly recommended. They provide, in a concise and clear way, an invaluable source of information which can provide a sound basis for further study.

The philosophy behind Arnold's *Studies in Biology* series (London) is an essentially sound one. The series provides a medium by which biologists can be kept up to date with developments in specific areas of biology. They are reasonably priced and are an ideal way by which individuals, schools, colleges and universities can supplement their libraries in particular fields.

Small rodents and insectivores have provided much of the data that form the basis of population and community ecology. In *The Ecology of Small Mammals* (£1.90; 95 pence) Dr M. S. Delany strikes a nice balance between discussing them as animals of interest in their own right because of their abundance, economic importance and role in the terrestrial ecosystem, and showing how they have contributed to general ecological theory. He covers a great deal of material, deliberately widening his subject matter to include species from as far afield as East Africa, America and the Far East. He does not attempt a comprehensive review of this material but aims to highlight the areas in which new developments are taking place.

The book begins with a discussion of some of the basic methods of trapping small mammals and of estimating their ages, home ranges and population densities. It then considers the life histories and demographic patterns found among these animals in different habitat types around the world. This is followed by a chapter on populations and the way they are affected by seasonal changes, food supply and predation. The final chapter deals with relationships between small mammal species and the way communities of species are related to different habitats.

Dr Delany's book is clearly and concisely written, well illustrated and fully referenced. Perhaps its greatest virtue is that it leaves the reader with the motivation to read further on the subject.

The student of zoology is generally brought up with the notion that animals are anatomically symmetrical, and great stress is laid on such characters as radial and bilateral symmetry as fundamental

features of different animal types. In *Animal Asymmetry* (£2.60, £1.30) Dr A. C. Neville points out that asymmetrical organisation is commonly found right across the Animal Kingdom. For each example he shows how asymmetry is adaptive in the life of the animal. Some insects have left and right wings of slightly different shapes so that they can be folded one over the other. Owls have differently shaped left and right ear apertures that enable them to locate sounds with great accuracy. Such asymmetries raise interesting questions in developmental biology and Dr Neville discusses briefly how they may arise during embryonic development in molluscs, insects and other animals. He also points out that asymmetry is a property of organic molecules and of the carbon atom itself.

Dr R. F. Chapman's book, *A Biology of Locusts* (£2.80; £1.40), should prove invaluable to any school or university laboratory that keeps a colony of locusts. It deals with the basic biology of these insects, their life history, feeding behaviour, flight and migration. The book also discusses the ecology of locusts in the wild, particularly from the point of view of their impact as serious agricultural pests. Finally, he discusses the various techniques that have been used to control locusts and also those that are now being developed. This is a very useful book because it contains, in a very readable and clearly illustrated form, virtually all that one could want to know about animals that are used very widely by students at all levels.

The aim of the series, *Topics in Biological Sciences*, published by Hutchinson (London), is to emphasise the social and economic importance of a number of biological topics. *The Biology of Insects* (£4.95; £2.95), by C. P. Friedlander, begins with an account of the structure, physiology and natural history of insects, along the lines that one finds in many biological textbooks. This is followed by a chapter on their behaviour and ecology and, finally, a chapter on the economic importance of insects and the methods used to control them. So wide is the scope of this book that its coverage of any one topic is of necessity rather superficial, and it is difficult to see that it represents an advance on existing entomology texts. *The Biology of Locusts*, however, in the *Studies in Biology* series, which considers only one species of insect, brings out much more successfully the way that an understanding of an insect pest's biology can contribute to its effective control.

Also in this series, *Freshwater Biology* (£4.95; £2.95), by L. G. Willoughby, similarly attempts to cover a very wide field. It deals successively, as most books on freshwater biology do, with the physical and chemical properties of water, with freshwater plants including

algae, and with a number of aquatic invertebrates and fishes. There is a brief chapter on features peculiar to running water habitats, and a longer one on decomposition cycles and nutrient recirculation. The chapters on social and economic aspects, such as water supplies, sewage treatment and pollution are very brief and serve only to outline these important topics. This book suffers, like its companion volume, from trying to cover too much material in too small a book. The attempt to combine basic biology with social aspects is not very successful; the latter seem to have been added almost as an afterthought. These books are not cheap and certainly not as good value for money as the *Studies in Biology* series.

These three series provide an interesting contrast. The authors of the *Oxford Biology Readers* are for the most part senior, distinguished biologists, and they have written with the authority one would expect on what are generally traditional biological topics. The *Studies in Biology* series are mostly written by a younger generation of authors, and their titles and style reflect a rather more progressive approach to biology. Both series can be highly recommended; they are cheap, well illustrated and well written. There is perhaps a risk that the *Oxford Biology Readers* will be used merely as 'essay cribs' by students; the *Studies* are more likely to stimulate an enquiring approach in their readers. The Hutchinson series has not been nearly as successful as the other two. Although well illustrated, they attempt to cover too wide a field in each volume, and their price brings them into competition with more thorough and complete textbooks, with which they do not compare very well.

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THE emergence of membranes as a major growth area in biological science has naturally provoked a proliferation of student texts in this area. These supplement the accounts to be found in general textbooks of biochemistry and cell biology and would therefore be expected to provide the integration and advanced information that these texts omit, but without too much repetition of readily available foundation material.

An attractive book, appropriate for students, is Weissman and Claiborne's *Cell Membranes: Biochemistry, Cell Biology and Pathology* (HP Publishing: New York, 1975, £12.00), but the price means that students are likely to read it only in a library. It is well written by eminent authors, but the multi-author format sometimes leads to repetition (for example, Rasmussen and Goldberg, Pappas and Loewen-



stein) and to an imbalance between the relative importance of a topic and the space allowed to it.

A student wanting his own membrane text then has a choice. Either he can get one of several small books that aim at a complete basic coverage of membranes, such as Saier and Stiles, Harrison and Lunt or a competitor (Quinn, *Molecular Biology of Cell Membranes*, Macmillan, 1976, £8.45; £3.85; Finean *et al.*, *Membranes and their Cellular Functions*, Blackwell, 1974, £2.80; Parsons (ed.), *Biological Membranes*, Oxford University, 1975, £5.00) or he can select his information piecemeal from series publications such as Oxford University Press' *Biology Readers* (London) or Chapman and Hall's *Outline Studies in Biology* (London).

Of the single texts, M. H. Saier and C. O. Stiles' *Molecular Dynamics in Biological Membranes* in the *Heidelberg Science Library* (Springer: Berlin and New York; DM 13.80; \$5.70) is most biologically oriented (despite its misleading title). The authors have a firm, maybe correct, unitary belief that membranes are all similar and that function invariably depends on structural fluidity; this sometimes leads to unsupported statements such as "(Inter-cellular) junction formation is undoubtedly dependent on membrane fluidity". Sometimes, they lose sight, however, of their unitary philosophy, as when sensory chemoreception (for example, taste) and hormonal chemoreception (for example, insulin receptor) appear in different chapters. These problems, and the limited and personal choice of topics discussed, makes the book unsatisfactory as a central student text. It does, however, include useful accounts of a number of membrane-mediated processes not normally treated in small texts (for example, bacterial chemotaxis, membrane fusion, and so on), provided the reader does not expect attempts at molecular explanations.

By contrast, R. Harrison and G. G. Lunt, in *Biological Membranes: Their Structure and Function* in the *Tertiary Level Biology* series (Blackie: Glasgow and London, £7.80 hardback; £3.90 paperback), have aimed at a systematic and balanced text with membrane chemistry as its dominant theme. The result is good but patchy. They are at their best when discussing "Membrane carbohydrate and cell surface specificity" and in a valuable and concise section on physical methods used in membrane studies. More than half of the remaining space is allotted to membrane, morphology, composition, isolation and structure, with a lot of space occupied by electron micrographs of doubtful educational value and by basic lipid chemistry (including

three illustrations of the same glycerophospholipid structures). This over-indulgence restricts the space available to membrane functions (transport, secretion, turnover, enzymes, receptors, and so on) even though they have equal billing in the title.

Membranes feature in the *Outline Studies in Biology* series (£1.30 each) in the recent books by A. M. Malkinson and by M. F. Greaves, in M. Davies' *Functions of Biological Membranes* (better titled *Membrane Transport*) and in a promised *Membrane Assembly*. Malkinson's *Hormone Action* presents twin discussions on hormone actions mediated through cell surface receptors and through intracellular receptors. This contrast is useful, and the material presented is up-to-date. Only about one third of the whole, however, relates to membranes. Greave's *Cellular Recognition* is an excellent text that draws together diverse phenomena (hormones, neurotransmitters, the immune system, tissue differentiation, sex in yeasts, and so on) and discusses them in terms of the reception and interpretation of environmental information at the cell surface. It is logical and well organised, with substantial sections on membrane structure, receptors, and mechanisms of stimulus-response coupling. A major virtue of series publications is that it can allow an articulate specialist to pass on his fascination with such a problem. But the series format also has drawbacks: the high cost of collecting the books needed to achieve a final patchy coverage, duplication of fundamental information on membrane structure, and sometimes poor editing and presentation.

Two *Oxford Biology Readers* fall into the membrane category, and are straightforward biochemical introductions to their topics. J. A. Lucy in *The Plasma Membrane* (45 pence), has packed a lot of basic information on membrane structure, turnover, fusion and transport (illustrated diagrammatically as an unlikely rotating macromolecule) into his 16 pages. Hormones are left to P. J. Randle and R. M. Denton's *Hormones and Cell Metabolism* (45 pence), a balanced and appropriate short account of metabolic control. This, however, has little on membranes other than control of adenylate cyclase, and is marred by inconsistency of units (insulin) and nomenclature (phosphorylase), repetition of diagrams and some almost illegible yellow-on-white printing. Other useful membrane-related titles in this series are *Lysosomes* (A. C. Allison), *The Golgi Apparatus* (G. M. W. Cook), *Mitochondria* (J. B. Chappell and S. C. Rees) and *Differentiation in Higher Plants* (D. H. Northcote).

If I could select only one item from this collection it would undoubtedly be Greave's *Cellular Recognition*: it wins both on scientific quality and value for money, even though it explores only one area of membrane function.

**Bob Michell**

*Bob Michell is Lecturer in Biochemistry at the University of Birmingham, UK.*

"Of making many books there is no end . . . Fear God, for this is the whole duty of man". Statements like this frequently explode from scientists drowned in a suffocating morass of reading—even though their theological intent may be different to the original Old Testament context. Nevertheless it could be an apposite comment about the eight books under review: all come from long and continuing series, four from Chapman and Hall's *Outline Studies in Biology* which includes 22 titles published since 1973 (London; distributed in USA by Halsted/Wiley: New York; three from Arnold's *Studies in Biology*—71 published since 1966 (London); and No. 72 in Oxford University Press' *Biology Readers* series, which began publication in 1971 (London)). All the series state that they are trying to fill gaps left by standard textbooks, either at the interface between traditional disciplines or in rapidly developing topics where textbooks are quickly out-of-date.

The Oxford series is the slightest in format, designed so that each one "will fit conveniently in a file among the students own notes". The series is specifically aimed at sixth-formers (school children at advanced level), students in Colleges of Education and Polytechnics, and first-year undergraduates. Each volume (really pamphlet—they have about 14 pages) covers a specific limited subject in reasonable depth and with good illustrations. J. L. Jinks's *Cytoplasmic Inheritance* (45 pence) is only one of 19 in the series devoted to genetics and evolution; like its companion volumes it has no bibliography but a list of "further reading", which includes five books and five reviews or key papers.

The other two series are directed at the second- to third-year university level, although they are in general free from jargon and thus potentially valuable to sixth-formers and their ilk. Chapman and Hall's *Outline Studies* (paperback editions only) cover subjects which may be expected to form a normal university course of 5–10 lectures. Arnold's *Studies* (hardback and paperback editions), on the other hand, tend to deal with topics that do not fall neatly into usual teaching slots, and for that reason seem to be more stimulating. For example, Bernard John's *Population Cytogenetics* (£3; £1.50) is

concerned with both animal and plant chromosomes; with population phenomena as well as straight cell biology; and with the quantitative effects of chromosome substitution. This enables the author to point out that knowledge is lacking about population cytodynamics and the effects of chromosome rearrangements on the genetical structure of populations—fairly complicated concepts which emerge naturally because of the interdisciplinary treatment of the subject. In contrast, D. M. Moore's *Plant Cytogenetics* (£1.30) and L. M. Cook's *Population Genetics* (£1.30) in the *Outline Studies* series, although both are excellent in their own ways (Cook in particular weaves mathematical formulation with natural history in a refreshingly non-intimidating manner), are almost inevitably more pedestrian. They will be useful for supplementing courses where no teacher is available for the topics considered (or alternatively, for providing unversed teachers with prepared teaching material).

One disadvantage of the lecture-course approach in a book is that it exposes gaps in treatment which would (or should) not appear in a comprehensive textbook and which would probably be filled by other teaching in a complete school or university course. For example, R. A. Woods in *Biochemical Genetics* (*Outline Studies*, £1.30) gives a useful summary of gene action and its control in microorganisms, but does little more than mention higher organisms. He justifies this limitation by claiming that "the information they [microorganisms] have yielded is 'cleaner' and more suited to an outline text". True, but this means that the title of the book is misleading. Again in the same series, A. McDermott gives a full introduction to human chromosomes and their variation in the *Cytogenetics of Man and Other Animals* (£1.30) (a topic which is only touched on in general biology or genetics texts), but *other animals* are only referred to in passing, largely in relation to human studies.

The *Studies in Biology* series is sponsored by the Institute of Biology, and this academic input may explain their imaginative choice of topics. One criticism that has been levelled at the series in the past is the relative lack of references in many of the volumes. In fact the three volumes from the series under review seem to contain enough key references for practical purposes. The *Outline Studies* series is variable: two of the present books have fairly extensive bibliographies, the other two merely a selection of "further reading".

Two other recent and relevant titles in the *Studies in Biology* series are *Genetics and Adaptation* characterised by E. B. Ford's usual lucid and idio-

syncratic style, and *Population Dynamics* by M. E. Solomon, a new edition of a useful book first published in 1969 and now fairly extensively revised.

One great advantage of limited topic books is that leaders of research in particular fields can write on their own topic: four of the eight under review are written by authors who have previously published substantial works on the same subject (Cook, Ford, Jinks and John). The *Studies* and *Outline Studies* series both work out at 2 pence per page in paperback; this is quite good value for money nowadays. It is difficult, however, not to hark back to the Penguin *New Biology* series which lasted from 1946 to 1960 and filled the gaps in textbooks so well at the time. But perhaps biology as well as publishing has changed . . . **R. J. Berry**

*R. J. Berry is Professor of Genetics in the University of London, UK.*

FEW books pack so many lessons for the plant physiological world as the slim volume on *Translocation in Plants* by Michael Richardson, No. 10 in Arnold's *Studies in Biology* series (£1.90; 95 pence). The book—now in its second edition and proclaimed an Open University Set Book—has already served many generations since first published in 1968. Why its popularity? It takes the form of six chapters with well defined subsections, almost suggesting it began life as a revamped set of lecture notes. Perhaps this explains its sales success, but also its failings?

Probably the most glaring error is the use of a water hammer (pp18–19) to measure the cohesive properties of xylem sap. Nowadays students see more mass spectrometers than Berthelot tubes or water hammers, and few would have sufficient knowledge to question explanations given. Dixon made the water hammer (1914) to illustrate cohesive attraction between water and glass in the presence of gas near vacuum. The experiment determining the limiting cohesive tension of water was carried out by Berthelot (1850) in a tube filled completely with water: both systems were described clearly by Dixon.

Phloem research has advanced rapidly in the past decade. Broadly the position is one in which the model proposed by Hartig and expounded more precisely by Münch (1930) has become established and received widespread acceptance. For centuries it was obvious that channels for dry matter translocation existed in bark of plant stems. After the discovery of sieve tubes the main question outstanding was why phloem sap should not flow through these pipe-like cells, carrying dry matter in solution to swell storage organs, ranging from the humble spud to the massive fruits of palms.

The main reason sieve tubes might not function like pipes, translocating the sugary sap, arose from the fact that at frequent intervals sieve tubes are occluded by sieve plates, the holes in which seemed to be plugged in both light and electron microscope studies. This observation gave rise to some alternative hypotheses and "the great phloem controversy". Most alternatives are faithfully reproduced and even extended in the new edition of the book. Yet considerable strides have been made in phloem physiology from studies of plants which, on wounding, exude phloem sap, as might be expected by Münch's hypothesis. Virtually none of this work has found its way into the new edition. There is some justification to withhold some of the detailed electron microscopy which has contributed to the widely held view that sieve plates serve a protective function, sealing rapidly when the system is injured. But the omission of this aspect so completely cannot be excused, because it is so central to the theme of the book. Nor is it legitimate to call aphid stylet sap "uncontaminated" (p36) on account of saliva injection effects (see, for example, *Biology of Aphids* by A. F. G. Dixon, *Studies in Biology* No. 44; 75 pence).

Another *experimenta crucis* which has contributed to the phloem controversy is the alleged demonstration of bidirectional transport in phloem. Certainly no sieve tube could, if it functioned as a pipe, conduct simultaneously in opposite directions at rates exceeding those of diffusion. But the experiment chosen to illustrate this, by Palmquist (1939), is dangerously misleading, as is obvious to anyone who has carried out even limited research on the system. The problem is that xylem pipes, containing sap under tension, lie in close proximity to the highly pressurised sieve tubes being investigated. Abraded leaves can rapidly suck solutions into xylem tubes producing an illusion of bidirectional transport. Much better research (for example, Turgeon and Webb, 1973) would have been desirable alternative material. Similarly much of the earlier work could have been omitted without loss. Sadly the five new pages and seven new paragraphs are largely (especially with hindsight) irrelevant.

The success of this book is a disturbing reflection on both scientific and publishing fraternities. If a better alternative of comparable cost existed, I hope students would have been guided to choose it in preference: such a volume is clearly lacking. If errors have escaped criticism so long, does this imply the capacity to publish extends beyond a proper degree of rigorous criticism? It is indeed regrettable that this book mars the high standards of the series as a whole. **John A. Milburn**

*John A. Milburn is Senior Lecturer in Botany at the University of Glasgow, UK.*



In the past two years, three publishers have made additions, in the field of muscle biology, to their (continuing) series of books designed for late school or undergraduate consumption. Oxford University Press (London), in their *Biology Readers* series, have produced a 16-page pamphlet entitled *The Contractile Behaviour of Mammalian Muscle* (30 pence) by A. J. Buller; Arnold (London) have persuaded D. R. Wilkie to write a second edition (hard and paperback) of his book on *Muscle* (£2.50; £1.25) in their *Studies in Biology* series; and Blackie (Glasgow and London) have produced a specialised text on *Visceral Muscle: Its Structure and Function* (£5.10) by H. Huddart and S. Hunt.

Muscles, according to Professor Buller and Wilkie and Doctors Huddart and Hunt, respectively, are more important in getting animals about than brains; are the means by which we act on our environment and move objects as well as ourselves; are a tissue and therefore by definition composed of cells. These paraphrased quotations from the first lines of the three books are characteristic and exemplify their approaches.

The books vary enormously in length and in their scope and coverage. Professor Buller concludes all he has to say in 16 succinct pages, of which 5 are taken up by illustrations. Wilkie covers his subject in 67 more sparsely illustrated pages, whereas Huddart and Hunt pack their comprehensive but more specialised subject matter into 165. The shortest work concentrates almost exclusively on isometric and isotonic contractions of fast and slow twitch skeletal muscles, having briefly introduced the subject by way of the ultrastructure and the nervous architecture of muscle. Professor Wilkie, as becomes a pupil of A. V. Hill, does not neglect the thermodynamics of muscle energetics, but also introduces the subject by means of the ultrastructure and chemical composition of muscles. In his book, he gives an overview, taking his examples from all sorts of muscle and from many species. He describes the common experimental methods used in examining muscles, pointing to their shortcomings. He explains the results obtained in terms of the known structure. He leads the reader rather gently through a clarification of the electrical phenomena in quiescent and stimulated muscle and illuminates the chemical changes that result in contraction. Finally, he brings us back to everyday experiences by describing how muscles maintain tone and enable us to take exercise.

The largest, although still slim volume, has a wider target for a narrower topic. It claims to be of interest to students of physiology, medicine,

biology and pharmacology and deals specifically with *Visceral Muscle*. It is the most conventionally organised of the three; once again, the contents run from microscopic structure through innervation to mechanical activity, but conclude with the protein structure and the molecular basis of contraction. Although some of the chapters are of course specifically oriented to visceral muscle, it is surprising how often comparisons are made with experimental work in skeletal and cardiac muscle.

Clearly, here is a triad of books that can be used quite differently, yet they are linked by a unity, since much of the material is common to the three. Wilkie's book stands out above others in the way that it simplifies complex ideas and yet has more than enough meat for any hungry student. Anyone remotely interested in muscle—and who among biologists is not—can—and one might almost say should—read it. The result will be not only an increase in knowledge and understanding, but an experience of an outstanding example of written communication. In addition, the author is a realist who has the interest of students at heart; he does not give the usual unmanageable list for further reading, but confines himself to a few seminal works.

Buller's contribution is more a pamphlet than a book; it probably contains all that a student need retain about the fundamentals of muscular contraction.

Most immunologists are preoccupied with problems pertaining to mice or men but many are now showing an increasing interest in the immunological responses of other species in order to discover evolutionary trends in recognition of foreignness, structure of antibody molecules and mechanisms of protective immunity.

In *Comparative Immunobiology* (£6.60; £3.30), in Blackie's *Tertiary Level Biology* series, Margaret Manning and Rodney Turner are the first authors to write a textbook of suitable size and clarity for students of immunobiology. Indeed, this book can be recommended to anyone seeking an introduction to comparative immunology. Other books have been and are being written on this subject but great credit must go to these authors for providing a book which will obviously remain a basic textbook on the subject for many years to come.

Those immunologists already involved with the vagaries and complexities of immunological responses in different species will admire the layout of the book. First, there is a 28-page introduction on the basic tenets of immunology, largely derived from work on mammals and birds. There follows

The question arises: is he more likely to retain nearly all of this or only a quarter of the slightly longer text? The answer must depend on the assessment of the teacher and the character of the student. It would be reasonable for any biologist or medical student to read a mere 11 pages, but how many have the time or the inclination to read ten times that many on a single subject? The beauty of this pamphlet is that it is short, lucid and expert, yet it does not have unrealistic expectations of the reader.

What then of the last book? It is not for every student; its primary use might be for reference, for each chapter is almost complete in itself, containing at least two key references. In addition, there are some 150 further references, and above all it is the only one of the three with an index.

It seems that these three complementary works offer three solutions to the problem of the modern science student—an elegantly produced evening's reading for tomorrow's seminar, a readable text from which something will be remembered, or the small reference for a narrow subject to be dipped into as required. You pay your money (0.30; £1.25 or £5.10) and you take your choice.

**Rainer Goldsmith**

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an ambitious description of immunological responses as we climb the 'evolutionary tree' from sponges to fishes and terrestrial animals. Each chapter is introduced by an accurate phylogenetic tree. Phyla which have been sufficiently studied by immunologists are lucidly described in clearly defined sections headed 'phagocytosis', 'transplantation reactions', 'humoral reaction and factors' and 'lymphoid tissues'.

One of the most intriguing aspects of invertebrate immunology is how animals with no lymphocytes can recognise foreign species. This is treated adequately here, but new theories on recognition now appear monthly.

The bursa of Fabricius is wisely described as being unique to birds (p149), and this should be a warning to those immunologists still hunting for a 'bursal equivalent' in mammals.

This is a concise book, small in appearance but large in its breadth of treatment and factual content. All immunologists should find time to read it.

**J. B. Solomon**

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## Embarking on biochemistry

WHEN, until recently, I lectured to large groups of science or medical students embarking on a course of biochemistry, I took along to the first lecture about 15 different texts. I tried to explain the merits of the various books in terms of the widely varying attitudes of the students. After the lecture many students would stand perplexed and some would even express their feelings to plead that they be told what to buy. I understood their viewpoint but I think, as teachers, we must resist the students' plea. The truth is, of course, that biochemistry is blessed with a wealth of excellent texts and the best-buy for any particular student is seldom an easy decision. The problem is made more difficult in the knowledge that unless a student buys a textbook by half-way through the first term he is unlikely to do so at all. In other words he has often to make his choice before he has much idea as to his interest in the subject.

The two texts under review are both excellent in their way. A. L. Lehninger's *Biochemistry: The Molecular Basis of Cell Structure and Function* (Worth: New York, second edition, \$17.50) is now clearly a classic and is extremely popular with students both of science and medicine. It is also comparatively cheap in terms of length and the excellence of the printing. *The Biochemistry of the Tissues* (Wiley: London and New York, second edition, hardback £14.50, \$30; paperback £6.25, \$13.50) by P. Banks, W. Bartley and L. M. Birt is a more modest effort, based on their lectures to medical students at Sheffield University.

A continual problem in the teaching of biochemistry is to keep the picture of the subject as a whole before the student. Too often the older courses appeared fragmentary and the student lost the unity of the subject while they delved into the minutiae of the various aspects. Lehninger keeps the story going by starting with a set of organising principles and continually referring back to them as he deals with various subjects. The story holds together very well and this is undoubtedly a main attraction for the students. The Sheffield authors had a particular group of students in mind and have tried to hold attention through a consideration of the biochemistry of the various tissues. The student has to be prepared for this approach, however, so that the earlier chapters are a somewhat skimmed traditional text.

In some ways *Biochemistry* by L. Stryer (Freeman: San Francisco) has much to offer medical students. In this

beautifully produced book reference is continually made to the relevance of biochemistry to medicine and physiology. Thus there is an excellent chapter on sickle-cell anaemia. Indeed there have been attempts to organise a course of biochemistry on a selection of such diseases. One thinks of *Biochemistry: A Case-Oriented Approach* (Mosby: Missouri) by Montgomery *et al.*; but again there are snags, for the student must have a proper groundwork before he is ready for this kind of treatment.

Another unifying approach that is now often used in first year courses is

to develop the biochemistry from an elementary study of cell biology. This is probably better than concentrating on the tissues. So far, however, I have not seen a textbook based on this kind of course.

In summary, both books reviewed have their strong points and certainly the latter part of the Sheffield book would prove a useful adjunct to the cheaper and much more comprehensive Lehninger text.

P. N. Campbell

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## Biochemical tools

*Physical Biochemistry: Applications to Biochemistry and Molecular Biology.* By D. Freifelder. Pp. x+570. (Freeman: San Francisco, 1976.) Hardcover £12.40; softcover £8.00.

A THOROUGH understanding of the physicochemical tools used by the modern biochemist is essential to their intelligent use. It is unfortunately a common experience of many, if not all, teachers of physical biochemistry, however, that one of the major obstacles in the path of a diligent student is the labour necessary to wade through the often complex and seemingly endless theoretical and mathematical derivations which represent the standard approach to the subject. One solution to this dilemma is to choose a limited number of techniques and to develop fundamental equations clearly from first principles with appropriate simplifications. Such an approach is justified by the quantitative nature of many of the techniques and has been used successfully by K. E. Van Holde (also entitled *Physical Biochemistry*, Prentice-Hall).

An alternative and in some respects radical solution is to adopt an almost completely non-mathematical approach. Professor Freifelder has done just this with the single aim of enabling "the student to understand and read the current literature". Equations cannot be avoided, of course, but although their derivations are, the physical principles, the assumptions behind the derivations and the conditions which must be satisfied for the equations to hold are clearly defined. *Physical Biochemistry* is therefore an exceptional book in that it neither requires nor contains a knowledge of basic thermodynamics which inevitably characterises other comparable texts. For this alone many students will be thankful.

Throughout the book, Professor Freifelder's overriding concern is to

stimulate the students' interest in the techniques of physical biochemistry, and if the avoidance of mathematical treatments is one cornerstone of his strategy, then the demonstration of the usefulness of the techniques in solving biological problems is the other. Indeed, the outstanding feature of his book is the wealth of biological examples illustrated. The majority of these are drawn from the molecular biology of bacteriophages and the physical biochemistry of DNA and viruses, reflecting Professor Freifelder's research interests and making a welcome change from the more conventional protein examples.

The subject matter is generally comprehensive, comprising an extensive section on direct observations by light and electron microscopy, general laboratory methods, separation and identification of materials, hydrodynamic methods including an especially well-written chapter on sedimentation, and spectroscopic methods including a useful introduction to nuclear magnetic resonance. The qualitative approach seems nevertheless to have led to some curious omissions for a text on physical biochemistry. Thus all scattering techniques are absent. In addition there is no treatment of multiple, small molecule binding. Surprisingly, a section on immunological methods is included.

This book is properly written for final-year undergraduate and first-year graduate students. Many of them will appreciate its non-mathematical approach from which there are undoubtedly great advantages of motivation to be gained. Ultimately, however, the student who finally arrives at the practical application of a technique for his own research purpose can only benefit from a thorough acquaintance with the underlying theoretical and mathematical analysis. This book will surely engender in the student sufficient enthusiasm to make the necessary effort.

G. L. Kellett

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## Endocrinology of steroid hormones

*Molecular Endocrinology of the Steroid Hormones.* By D. Schulster, S. Burstein and B. A. Cooke. Pp.xv+321. (Wiley: London and New York, 1976.) Cloth £9.75, \$20; paper £4.75, \$10.

THIS textbook on molecular endocrinology of the steroid hormones is an attempt to focus attention on the mechanism of action of steroid hormones. Its aim is to provide broad coverage with the minimum of fine detail in four main sections—methods, the endocrine glands, regulation of biosynthesis, and mode of action of steroid hormones.

The book is not intended to be a reference text for research workers, but is an account of steroid hormone biosynthesis and mode of action aimed at science students perhaps majoring in biochemistry, or at medical students. There is no doubt that a science student with a reasonable background in chemistry and biochemistry could read this text with ease.

The methodology of steroids is briefly reviewed with a fairly cursory treatment of radioimmunoassays. It might have been appropriate to have given this aspect of methodology a more extensive treatment. If the text is to be used by preclinical medical students then it is likely that most of the steroid hormone assays which they will request as practising doctors will be carried out using this method. A detailed account of the technique including its shortcomings might have been to the reader's advantage.

Biosynthesis of steroid hormones is presented in a fashion which will be acceptable to undergraduate science or medical students. Short chapters deal with the structure and function of the pituitary, hypothalamus, adrenal cortex, ovary, testes and the fetal-placental unit. These chapters each contain a brief account of the structure and ultrastructure of the endocrine organ, its blood supply and its relationship to other tissues in the body. This section will be extremely useful to students from the physical sciences who wish to grapple with the concepts of endocrinology.

The third section of the book, which is concerned with the regulation of steroid hormone biosynthesis, contains three chapters, the largest being the control of corticoid synthesis by ACTH. Problems associated with the acceptance of the usual dogma on the mode of action of ACTH are critically reviewed in this chapter. This is followed by a brief treatment of aldosterone synthesis and a short chapter on the control of androgen and

oestrogen secretion by FSH and LH. In view of the considerable interest at the present time on the control of FSH and LH secretion, I would have preferred to have seen a more extensive treatment of the subject. For example, organic chemists and other non-biological scientists in the pharmaceutical industry involved in work associated with contraceptive preparations could profitably read all of this book, but this section, which would be of great interest to them, is barely seven pages in length.

The production of even a short textbook involves a considerable gestation period. The last section dealing with the mode of action of oestrogens, progestins and androgens covers topics which are currently under active investigation. Most of the references contained in this section are to papers published pre-1975; because much work has emerged in 1975 and 1976, the medical or science students reading these chapters will be obliged to update the information. The basic concepts of the mode of action of steroid hormones, the localisation of the receptors and the possible nuclear events are constantly undergoing refinement and revision. These chapters are superficial, but they will whet the appetite of the

student; and the enthusiast will go on to read beyond this text.

It is regrettable that the mode of action of aldosterone does not receive a more detailed treatment. This is one of the few examples in steroid endocrinology where the investigator can in fact obtain a fairly precise physiological indicator of the action of the hormone in an *in vitro* system.

This treatment of the endocrinology of steroid hormones will be welcomed by many teachers. The textbook will be valuable to students who are reading biochemistry either for a general degree or for an honours degree, and many of the problems associated with the interpretation of the mode of action of steroid hormones are highlighted. Since the subject is so wide the treatment is superficial in parts; but it is always interesting, readable and relatively free from errors. The book can be recommended as a straightforward, clear account of the concepts of molecular endocrinology of steroid hormones in the mid-1970s.

G. S. Boyd

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## Introducing prostaglandins

*Prostaglandins: An Introduction to their Biochemistry, Physiology and Pharmacology.* By P. B. Curtis-Prior. Pp. xi+158. (North-Holland: Amsterdam, New York and Oxford, 1976.) Dfl. 42; \$16.25.

THIS volume is intended as an introduction to prostaglandins for those "currently uninitiated in the subject". The author begins the main text by describing the nomenclature and biosynthesis of prostaglandins. I found the latter section rather misleading in parts—for example, the transformation of endoperoxides to prostaglandins is depicted (wrongly) as a reversible reaction. Sections covering prostaglandin catabolism, inhibitors of biosynthesis and receptor antagonists, are followed by a chapter entitled "Prostaglandin-like substances" (mainly polypeptides), which I felt did not really belong in the book at all. The remainder of the text largely comprises an account of the effects of prostaglandins in physiological and pathological processes, although little was said about their role in the control of parturition or platelet aggregation—surely two areas where the case for prostaglandin involvement is stronger than most? The

final chapter deals rather perfunctorily with the important topic of biosynthesis of thromboxanes and other non-prostaglandin products of arachidonate.

The text is produced by a process of direct photographic reproduction of the typescript, and the difficulties this causes in the arrangement of the figures give this (otherwise well produced book) a slightly unbalanced look. I found the quality of the artwork rather variable, mainly because half of the figures are reproduced from other sources; a practice which leads to trouble in the final chapter where the wrong structure of thromboxane  $A_2$  is given.

This book is easy to read, but I found the chapters rather superficial and had the impression several times that the author was a little out of touch with the mainstream of prostaglandin research. There is, however, no "primer" on prostaglandins to which the "uninitiated" can refer, and no doubt many will be glad to find this information collected in a basic text. The book contains adequate references for further study.

R. J. Flower

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## Enzymes and cellular metabolism

*The Enzyme Molecule.* By W. Ferdinand. Pp. xvi+289. (Wiley: London and New York, 1976.) Cloth £9.50, \$20; paper £4.50, \$10.

THE study of enzymes is often regarded as being an end in itself rather than being an essential step towards an understanding of the way in which the cell functions and regulates itself as a biochemical entity. This book makes a valuable contribution to setting the study of enzymes in the wider context of cellular metabolism. The book, which is intended for undergraduate students, requires little initial biochemical knowledge from the reader, although some elementary mathematical ability is assumed.

The topics of simple thermodynamics, single-substrate enzyme kinetics, enzyme purification and protein structure and chemistry are dealt with in some detail in the earlier chapters and an appendix of the book. These chapters lay a solid foundation for the discussion of enzymes which occupies the remaining two thirds of the book. A chapter on enzyme structure and function covers the measurement of ligand binding, determination of residues at the active site, conformational changes in proteins and mechanisms of enzyme catalysis. This last section provides a clear and readable account of transition-state theory which should provide an excellent introduction to those wishing to take the subject further. The author has chosen to consider a single enzyme, ribonuclease, as an example of the chemical mechanism of enzyme action, and the account presented provides a good summary of the evidence which has led to current theories of its catalytic mechanism.

The effects of inhibitors, activators, pH and temperature on enzyme activity are covered in a single chapter occupying some fifty pages, which also includes a treatment of the kinetics of enzymes using two substrates. The attempt to cover such a wide range of topics in a relatively short space has led to a rather uneven treatment. The effects of pH and temperature on enzyme activity are given rather brief and superficial treatments, whereas the discussion of the Wong and Hanes method for deriving kinetic equations is excellent. In the consideration of the kinetic mechanisms of two-substrate enzymes the author's prejudices do not always coincide with those of the re-

viewer and generally this section concentrates upon nomenclature to too great an extent without considering the underlying causes of the observed kinetic effects. Cooperativity and allostery are considered in a separate chapter which makes a clear distinction between these two phenomena and discusses the major models of cooperativity and the kinetic predictions that they make. Aspartate transcarbamoylase is chosen as an example of an allosteric enzyme for detailed discussion and the author provides a lucid account of a somewhat confusing situation.

The first chapter of this book considers the role of enzymes within the cell and this topic is returned to in the final two chapters which discuss the role of enzymes in the control of metabolism. One of these chapters provides a somewhat brief outline of the factors affecting the control of enzyme concentration in the cell, whereas the other is an account of the kinetics of the regulation of metabolic pathways. This chapter provides an excellent account of how the enzyme kinetic theory considered earlier can be applied to the regulation of metabolic pathways within the cell. It should be required reading for all those who believe that the study of enzyme kinetics is an end in itself.

An important omission from this book is that there is no treatment of the kinetics of the transient phases of enzyme reactions. The author has obviously had to be selective in his choice of topics in order to deal with the study of enzymes in a relatively short space, but the lack of any treatment of this topic restricts the value of the book as a general introductory text on enzymology. Shortage of space has also led to a somewhat cryptic presentation of some topics such as X-ray crystallography and experimental methods for studying ligand binding. In addition the author occasionally makes categorical statements that should require qualification. For example, it is asserted that the initial rate of enzyme-catalysed reactions is always proportional to enzyme concentration whereas there are in fact a number of exceptions to this and workers should be encouraged to check for such linearity rather than just assuming it.

Despite the above reservations this book provides a good introduction to the study of enzymes and should prove valuable to many students, particularly in showing how this topic relates to the wider fields of biochemistry and cell biology.

**K. F. Tipton**

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## Differing approaches to genetics

SINCE a knowledge of genetics is now considered an important ingredient of many undergraduate courses, as widely disparate as molecular biology and sociology, very different approaches to the subject are to be expected. This is reflected in several undergraduate texts which have appeared in the past year.

M. I. Lerner and W. J. Libby's *Heredity, Evolution and Society* (Freeman: San Francisco, second edition, \$13.95; £8.80) is concerned more with the social consequences of genetics, although it would be unfair to suggest that the authors have neglected the scientific basis of the subject. The emphasis throughout is on the relationship between genetics and evolution, particularly human evolution. Yet though largely, but not exclusively, concerned with human genetics the text is not over-burdened with obscure clinical references which often reduce the appeal of such texts to science students. The standard arrangement of most student textbooks of genetics is not followed. For example, chromosome structure and function are not dealt with as such but are included in chapters on gene interaction, sex and mutation. An attractive feature of the book is the use of inserts, called 'boxes', in which are segregated most of the descriptive, biographical and tabular material. This is a clearly written text which is eminently readable.

Many biologists are daunted by genetics because of the emphasis on statistics. D. T. Suzuki and A. J. F. Griffiths recognise this problem, and their book, *An Introduction to Genetic Analysis* (Freeman: San Francisco, \$13.95) "... attempts to teach the reader how to do genetics". This is therefore not strictly a textbook, for it is not just concerned with concepts but is more a guide as to how to analyse genetic data. The approach is essentially quantitative, although a level of mathematical sophistication no greater than simple algebra is required. It is clearly meant to be a didactic text, extensive use being made of a question-and-answer format, problem-solving exercises (a separate book of answers is available), and the dispersal within the text of so-called "messages" which summarise important information. There are also sequences of diagrams illustrating mitosis, meiosis, DNA replication and translation which can be animated by letting the pages flip over. This latter device is not really very satisfactory and seems out of place in a University text. Quantitative problems in all aspects of genetics are considered including Mendelian segrega-

tion, linear and circular linkage analysis (but not lod score methods), cytogenetic mechanisms and mutation. There is also a chapter on population genetics. Perhaps it is in this latter field that mathematical analysis has been most important, yet this is given relatively little attention. The book is wide ranging in the material chosen to illustrate problems in genetic analysis, but it is difficult to see how it will survive competition with more conventional texts.

N. V. Rothwell's book *Understanding Genetics* (Williams and Wilkins: Baltimore, \$14.95), is perhaps the most conventional of these texts. It deals in the usual manner with the mechanisms of gene action, cytogenetics, various aspects of biochemical genetics and population genetics. The molecular basis of inheritance is dealt with very well indeed but rather oddly is relegated to the latter half of the book. This text provides a straight-forward account of genetic mechanisms illustrated by examples from widely related organisms. The illustrations are simple straight-forward line drawings which are very attractively presented. Each chapter concludes with some well chosen references, although very few of these are more recent than 1970. Apart from the chapter on probability there are no student exercises.

W. Hexter and H. T. Jost's *The Science of Genetics* (Prentice-Hall: Englewood Cliffs, New Jersey, £11.90; \$18.95) is also an introductory text designed primarily for biology students, but here the authors have tended to

use a historical approach in order to emphasise the reasoning which initiated critical studies and lead to important conclusions. The hope is that the book will have a two-fold purpose: "... to introduce the student to the essential facts of genetics and to introduce the student to the methods of science". In general the authors have been successful. Again, illustrative examples are widely chosen from both the plant and animal kingdoms. Of student texts which deal primarily with the science of genetics, this is perhaps the most readable and exciting, probably because of the emphasis on the evolution of concepts and ideas. It also becomes quite clear where there are still some major gaps in our knowledge and understanding, as in the case of chromosome structure. The book is enhanced by simple straight-forward line drawings and with references to key papers. Each chapter concludes with a number of exercises but no answers are provided.

These four books therefore reflect very different approaches to genetics. For those whose main interest lies in human problems and the social consequences of genetics, Lerner and Libby's book can be unreservedly recommended. For those who want a more standard undergraduate text of general genetics, Hexter and Jost's book is perhaps the best.

Alan E. H. Emery

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## Abundant facts of immunology

*Basic and Clinical Immunology*. Edited by H. H. Fudenberg, D. P. Stites, J. L. Caldwell and J. V. Wells. Pp. 653. (Lange Medical Publications: Los Altos, California, 1976.) \$12.50.

THIS large book is good value for money. Its intent is to act as a source from which the enquiring mind may obtain the abundant facts of immunology. There are impurities in this spring of knowledge some of which should disappear in the promised biennial revisions. The editors solicit our specific advice on this point.

The tone of the book is authoritarian and the referencing is inadequate to check the origins of many of the statements made. This may be in line with the current teaching of medical students but it is irritating for the more curious reader. The indexing is capricious, which is probably inevitable in a book

with forty chapters written by many different people. The line drawings are first class but the reproductions of photographs are in the main of indifferent quality.

The first half of the book deals with the basic aspects of immunology starting with a delightful chapter by Grabar on the historical background. The short chronology on important achievements in immunology, which terminates this particular piece, ends at 1959 at a time when many might think immunology was just beginning to gather steam.

The second half of the book starts with a summary of the methods in common use in immunodiagnosis and concludes with a series of chapters on clinical immunology.

The book is fascinating and reminiscent of Florey's texts on general pathology. My reservations simply concern the difficulty of teaching rapidly changing subjects without perpetuating too many errors.

A. J. S. Davies

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## Developing biologists

*The Developmental Biology of Plants and Animals*, edited by C. F. Graham and P. F. Wareing (Blackwell Scientific: Oxford and London, £7.75; Saunders: Philadelphia), is unusually courageous for a textbook in treating development as a biological problem, not simply as zoology or botany. This approach has clear gains (for example, cell interactions and control of differentiation) but also losses: a selection of topics that "have a bearing on general themes of plant and animal development" (my italics) inevitably excludes many of relevance to only one or the other. On the animal side, the book has nothing on extraembryonic membranes and, more seriously, next to nothing on cell movements.

The book is in six parts, most with an introductory chapter and short conclusion by the editors. Individual chapters are by 20 different authors, mainly writing from British laboratories (except J. T. Bonner from the USA). Integration is achieved by extensive cross-referencing. Figures are used sparingly, giving us a lot of text for our money. The book is advanced, not introductory: most chapters assume basic background, and some use an eclectic range of examples and terminology that would soon baffle a beginner.

In a book of this scope an individual is bound to have both praise and grumbles over facts or emphasis. Many chapters seem to me admirable critical summaries of our present knowledge—those on animal cell junctions and the control of gene expression are especially clear. On the other hand, the emphasis of the chapter on "self-assembly" seems misplaced. There is lots of physical chemistry, but very little on regulation, surely the central issue for developmental biologists. "Pattern formation in animal embryos" is not for the unwary, reading simply for information: it suggests short range inductions rather than gradients or positional information as the basic mechanism—a stimulating article, belonging more in a journal than a textbook. The section on environmental effects (rarely mentioned in accounts of animal development) disappoints me. Although most work has been on model animals in laboratories, emphasising the constancy of developmental processes, information is now appearing on ecological factors (for example, on the timing of amphibian metamorphosis), but none of this is mentioned.

Some small points: a student ignorant of *Ascaris* might get the

impression that chromosome diminution never occurs (p302). Is vertebrate limb elongation "entirely due to addition of tissue at the tip" (p180)? Why no mention of Roth's (1973) hypothesis as a mechanism of cell interactions (chapter 3.1)? And two irritations: tubulin repeatedly spelt tubullin (p259): reference lists at the end of each chapter, some in alphabetical order, others not, rather than altogether at the end.

Do advanced students need a textbook? Many courses prefer directed reading in the original literature and reviews, since ageing is rapid in the subjects covered here. Although the cover date is 1976, the book's literature survey just scrapes into 1974, comparing unfavourably with its most direct competitor, Lash and Whittaker's *Concepts of Development* (Sinauer: Stamford, 1974), almost as up-to-date, despite its earlier publication. The incompleteness makes the book less than ideal for either botanical or zoological courses (still in the majority?). Students

may simply regard the 'irrelevant' chapters as money down the drain.

Despite these general doubts, and a few grumbles about details, I'd still heartily recommend this book for the excellence of many of its chapters, for its thoughtful overall philosophy, and for those sections which include examples from both kingdoms—they'll make biologists of us yet!

I cannot so easily recommend John McKenzie's *Introduction to Developmental Biology* (Blackwell Scientific: Oxford and London, £4.25; Halsted/Wiley: New York). This takes the view that students need to be cajoled into an interest in development by examining human development first. This may be true of medical students, but not, in my experience, of biologists. For an introduction, the book has too many unexplained anatomical terms (no glossary) and minor errors.

**J. R. Downie**

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## Recipe books for crop production

*Crop Production: Principles and Practices* by S. R. Chapman and L. P. Carter (Freeman: San Francisco, \$15) is one of several similar books that outline the production of crops in the United States. All of them profess to introduce the principles of crop production before detailing the growing procedure for the major crops. For example, in this book by Chapman and Carter . . . "Our philosophy has been that modern highly productive farming practices depend on an understanding of the principles of plant growth. When the principles are grasped, the practices follow logically." This is a commendable philosophy for the teaching of undergraduates in agriculture, but when a closer look is taken at these introduction principles (approximately half of the book) we find, in spite of a beautifully illustrated text, a rather thin veneer of elementary botany covering once again mitosis and meiosis, simple plant morphology, properties of soils, and so on which seem too common an occurrence in so many agricultural textbooks that try to cover too much material. To give two examples, where are the principles relating economic crop yield to plant density, which can explain seeding rate and crop establishment practices; and where are the principles of fertiliser response curves on which all fertiliser recommendations need to be based?

As our present knowledge of field crop physiology gives a clearer understanding of many common crop practices throughout the world, it should now form the basis of crop production textbooks.

The second half of the book deals with production practices for each crop in the form of a growers' manual. This may be a useful source of reference, but one hopes that present undergraduates are not expected to learn and inwardly digest the factual details of crop-growing procedures. A further indication of thin coverage, is the allocation of only six pages of text to the maize crop, particularly since the crop originates from a country that produces 40% of the world's production.

In contrast, the latest edition of *Principles of Field Crop Production* (Collier-Macmillan: London, £7) by Martin, Leonard and Stamp gives some five times this coverage and would provide a better reference source. But in the final analysis, the recipe book approach to crop production makes this type of textbook no more effective than a farmer's manual for the country of origin. How, for example, does a University level agronomist, who has been reared on such a diet, adapt to unfamiliar environments, such as the third world, without an understanding of the principles of field crop growth?

**G. M. Milbourn**

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## Microbial physiology . . .

*Microbial Physiology.* (Basic Microbiology, Vol. 4.) By I. W. Dawes and I. W. Sutherland. Pp. v+183. (Blackwell Scientific: Oxford and London, 1976.) £4.25.

MICROORGANISMS can do so many things and can do some of them in so many different ways; consequently it is hard to give a short introductory account of microbial physiology which retains clarity and precision while covering a representative, if not comprehensive, range of material. The choice of topics in this book is judicious, but the coverage of them is somewhat uneven.

The first chapter is a concise account of the structure and organisation of both eukaryotic and prokaryotic microbial cells with a useful digression on flagellar function. This is followed by a chapter on the growth of cells and populations. Although the cell cycle and its relation to both production of new cell surface and DNA replication are well described, the part on growth of batch and chemostat cultures is rather slight. A discussion of cell death and the mode of action of some antimicrobial agents is, however, included.

The third chapter, "Substrate entry into the cell", prefaces four chapters on microbial biochemistry. Energy production in heterotrophic organisms is covered in reasonable detail; and this is followed by a description of photosynthesis which is the worst part of the book, as it is not a particularly clear account, including an odd choice of detailed information and some unfortunate errors. Equally, the chapter on monomer biosynthesis is patchy. Some anaplerotic sequences are described in good detail, but it is not possible to deduce, for instance, how an organism might grow with succinate as sole carbon source. The choice of biosynthetic pathways and their description on the other hand makes pleasant and informative reading.

The next two chapters on macromolecular synthesis and the control of metabolism are also well done in general. The concluding chapter is concerned with morphogenesis, showing the importance of this sort of phenomenon for microbiologists and what marvellous experimental systems microorganisms can provide for the study of a variety of morphogenetic problems.

The standard of production of this book is fair: the print is rather small but the text is divided by frequent sub-headings. There are plenty of illustrations, most of which are informative,

although they vary in style and precision. Thus, the effect of temperature on the growth rate of *Escherichia coli* is shown as an Arrhenius plot with fully described scales and with data points (which is good), whereas the induction of  $\beta$ -galactosidase by isopropylthiogalactoside is shown as a sketch graph without scales or data points, and is actually incorrect (which is bad).

The further reading suggested also leaves something to be desired. Two to five review or textbook references are given for each chapter, but these do not always cover enough material. For

## . . . genetics . . .

Two recently published books on microbial genetics, aimed at first- and second-year undergraduates, cover essentially similar material, which can be broadly described as the organisation, function and recombination of bacterial and viral chromosomes and the molecular basis of gene action. The emphasis of the two books is, however, very different. About 30% of D. M. Carlberg's *Essentials of Bacterial and Viral Genetics* (Charles C. Thomas: Springfield, Illinois, \$24.75) is devoted to an interesting account of mutation and a further 30% to recombination systems in bacteria and viruses. About 40% of the new edition of J. R. S. Fincham's *Microbial and Molecular Genetics* (Hodder and Stoughton: London, hardback £3.95, paperback £2.25; Crane, Russak: New York), on the other hand, provides useful summaries of gene action, regulation, plasmids and DNA technology. Fincham follows the now traditional sequence of the properties of DNA, chromosome mapping and mutation followed by the topics already mentioned, whereas Carlberg covers protein synthesis, regulation and mutation before basic features of genetic systems which inevitably results in a degree of cross referencing.

As an introductory text Fincham's book suffers from not being self-contained. Some of the basic material on chromosome mapping and bacteriophage biology is not clearly explained. For example, it is not very helpful to provide ascus analysis data from a four-point cross in *Neurospora* together with a bald statement in the text that "Table 2 illustrates the principle" (p31). It is also difficult to understand why there are no diagrams of the lytic and lysogenic cycles of bacteriophages when there are diagrams of life cycles of *Neurospora* and yeast, organisms covered in only a minor part of the book. Another topic which lacks diagrams is the mechanism of sex factor insertion and chromosome transfer in

chapter two, there are no references on population dynamics and asynchronous culture techniques, although three references to cell cycle literature are given.

This book may be of use for microbiologists in the early stages of an undergraduate course and as a concise survey for students of biochemistry and genetics. I think it is poor value for money at its present price.

C. F. Thurston

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bacteria. In the preface the author hopes that the book will not be beyond the "ambitious beginner" in biology but one might add to that the "intelligent beginner", as I do not feel that this is the book to guide the average student through these aspects of genetics. Although the author provides selected textbooks and original articles for advanced students he does not really cater for elementary students working at this level. Reference to articles in *Scientific American*, *Science Progress*, *Science*, *New Scientist* and the *British Medical Bulletin* would have been more appropriate.

Carlberg deals with his subject matter rather broadly and includes some interesting material on industrial and medical applications of microbial genetics. His style is essentially that of a lecturer giving an outline of the material but "leaving much fine detail for the interested student to seek out in the literature". This approach has much to recommend it as the book is readable, interesting and clear, but it has the disadvantage that the book cannot be used to provide the detail usually expected in a course text.

Carlberg's book is more lavishly produced, which is reflected in the price. The diagrams, many of them original, are on the whole clear but some are below the standard expected. For example, the drawing of an icosahedral virus on p286 is inadequate, the models for recombination are not good, and a diagram on p193 shows a diploid cell with a haploid chromosome number. It is also surprising to find no mention of bidirectional replication of DNA. The inclusion of a patent application is interesting but it is difficult to see the justification for the inclusion of ten pages rather than an abstract of the important points.

Neither book attempts to be fully up-to-date in controversial areas, such as molecular mechanisms for recombination, so that the material included should not date too rapidly. It must be recognised, however, that most genetics texts only have a useful life of a few years. Neither book can be strongly

recommended for use as course texts although Carlberg's might be suitable for particular types of courses. Its price, however, would prevent its purchase as a standard text. For students studying genetics as well as microbial genetics, a more sensible purchase would be the second edition of M. W. Strickberger's *Genetics* (Collier-Macmillan: London, £6.30+0.95 pence for answer manual).

**Bacterial, Phage and Molecular Genetics.** (Springer: Berlin and New York, DM23; \$9.50). a practical manual by U. Winkler, W. Rüger and W. Wackernagel, translated from the German, is a very useful collection of experiments which complement well the manuals already available. There are a range of experiments on the purification of bacteriophages as well as detailed schedules on density gradient centrifugation techniques. Molecular aspects include the isolation and analysis of DNA from bacteriophages as well as experiments on mRNA. The use of ultraviolet light, one of the safer mutagens, is fully exploited to show lethal effects, mutagenesis and the induction of lysogenic and colicinogenic bacteria. Standard

bacterial and bacteriophage genetics experiments are included as well as transfection of *Escherichia coli* sphaeroplasts with  $\lambda$  DNA. The final section deals with gene action and phenotypic expression with systems ranging from morphogenesis in T4 bacteriophage to regulation of  $\beta$ -galactosidase. In addition to schedules for 25 experimental areas there are also brief introductions to background theory, useful references, five sets of problems and answers and (particularly valuable) 48 pages of the results expected from the experiments described.

This manual should prove very useful to lecturers attempting to broaden the range of experiments in microbial genetics courses. The book is clearly produced and usefully illustrated with graphs and specimen data sheets. The experiments described are not as ambitious as some of the recent manuals on molecular genetics but this has the advantage that most of the experiments should be feasible in a department with average equipment.

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## ... and ecology

**Interfaces in Microbial Ecology.** By K. C. Marshall. Pp. x+156. (Harvard University: Cambridge, Massachusetts, and London, 1976.) £9.40.

PROFESSOR MARSHALL has written a book that is attractive to read, and which is concise, selective, and clearly written. Microorganisms living at interfaces do so in a peculiar habitat; they are faced by unusual physicochemical stresses and must live a very different life from that of the bulk phase organism. What then do we hope to learn from a book that introduces us to the microbial ecology of this habitat?

The history of physical chemistry abounds with studies on the properties of interfaces; there are also clear ecological distinctions between gas-liquid, gas-solid, and liquid-solid boundaries; living bacteria exist in all environments and so must encounter such interfaces regularly. Topics of this sort are fundamental enough to need careful definition and discussion in an introductory text. Professor Marshall does precisely this, guiding us adeptly through some fairly advanced physical chemistry in the first few chapters, being careful to define his symbols as he does so.

Later chapters are concerned with "non-specific interfacial interactions in aquatic and terrestrial environments" and "specific interfacial interactions in

microbial ecology". Although some workers may not be convinced of the need for this distinction, Professor Marshall's treatment of aquatic and terrestrial environments is stimulating and provokes one to further research. He discusses the nature of bacterial adhesion, the problem of nutrients at surfaces, and the habitat of sedimentary microorganisms, and considers such terrestrial topics as moisture content, the rhizosphere and mineral dissolution. In his final chapter Marshall comments on the characteristics of oral and intestinal microorganisms, and lists some other microbial curiosities all of which prove entertaining.

Technically, the book is well produced, and the typographical layout is clear. Almost all of the figures and tables serve to amplify the text, except perhaps Fig 1.1 and 5.6, both of which suffer from being reproduced on matt paper. The bibliography is representative and the only fault I could find in the text was unfortunately a reference to my own work with Professor Crisp, which I thought was slightly irrelevant in a paragraph on primary microbial films (p61).

In general, I found Professor Marshall's book easy to read systematically, as well as being enjoyable to dip into, and can recommend it to advanced undergraduates and postgraduate research workers.

**P. S. Meadows**

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J. McKenzie M.D. 1976. 224 pages, 112 illustrations. Paper, £4.25

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Eighteenth Symposium of the British Ecological Society, edited by J.M. Cherrett and G.R. Sagar. April 1977. 350 pages, 100 illustrations. £14.00

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Studies in Ecology, Volume 4, by P.H. Nye M.A. and P.B.H. Tinker M.A. Ph.D. June 1977. 400 pages, 150 illustrations. About £12.00

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## Pocket guide to practical genetics

*Methodology in Medical Genetics: An Introduction to Statistical Methods.* By Alan E. H. Emery. Pp. 157. (Churchill Livingstone: Edinburgh, London and New York, 1976.) £6.50.

THIS is a compact little book (I have reviewed it bit-by-bit, carrying it around in my pocket), clearly printed and well produced. The style is painstakingly explicit, missionary in tone, and unpretentious in its objectives and exposition.

The scope might fairly, and with no slight, be described as a compilation of those methods which Professor Emery, a practical human geneticist of extensive experience, has himself used. They comprise: standard segregation analysis (including some of the newer methods not covered in the classical texts); linkage analysis with and without the Bayesian modification; principles, but not details, of genetic counselling; twin studies; quantitative methods; and some of the elements of population genetics. There are many tables of wide utility, at present eclipsed by the computer, but (if our grants remain unfunded) likely to undergo a revival.

The author's unwavering practicality spares us the pages of pettifoggish middle-grade mathematics which to the eyes of mathematicians and non-mathematicians alike, blight so many books on analytical genetics. His practicality is also a weakness. A really good mathematician with a true desire to communicate to the non-expert would have produced a somewhat more illuminating book. Here there are no underlying statistical principles stated: methods are produced with no visible means of support. Maximum likelihood estimation (MLE) is used, but we are told nothing of its properties, a neglect which has its counterpart in some of the methods chosen. For instance, there seems scarcely any point now in the Lenz-Hogben method which has never been well defended (for example, as to the asymptotic normality of the test statistic), is not based on a sufficient statistic, and falls far short of maximal power. On the other hand the MLE method recommended for the frequency of a rare recessive gene is so badly biased as to merit a jack-knife correction; in small samples it far from follows a normal distribution, a property implicitly invoked on page 5. (A Poisson approximation with confidence limits based on the  $\chi^2$  distribution would have been preferable.) Again MLE of  $\theta$ , the recombinant coefficient, is so biased for large  $\theta$  as to make estimation of loose linkage hazardous

in anything but immense samples: thus it scarcely matters what, if any, mapping function is used.

These defects are likely to leave the student, nurtured on this book alone, with little statistical sense and without either inventiveness or resourcefulness. This trend shows up in the method recommended for estimating the frequency of an X-linked recessive gene, which discards all information on female subjects. (Numerical MLE is straightforward, if a trifle inelegant.) The ABO problem is dealt with by Bernstein's method, which is absolutely efficient asymptotically but which would not be so for four alleles or more.

## Integrated text on population biology

*Population Biology.* By Thomas C. Emmel. Pp. xii+371. (Harper and Row: New York and London, 1976.) £11.95; \$15.95.

As the author remarks in his preface, "part of the problem of writing a book on population biology involves the host of inter-relationships among the basic contributing fields of ecology, genetics, evolution and behaviour". Dr Emmel copes well with this problem, providing a clearly arranged up-to-date account for the "well-prepared, advanced undergraduate". The book would also serve the professional biologist needing to catch up with some aspects of this recently integrated subject.

The chapters deal with cycles of matter and energy, population genetics and evolution, population dynamics, dispersion, dispersal, age and sex structure of populations, life-history patterns, mating systems, behaviour, climatic effects, and interactions of populations in communities. The reference list of 1,400 or so items is strong on recent contributions, and the index is reasonably comprehensive. The illustrations are on the whole excellent, although a few of the line drawings are disproportionately tall, and several of the half-tone pictures fall short of their purpose: the zebras in 11-14 do not appear cryptic, whereas the cicadas in 8-2b do (but should not).

In a fairly advanced book presenting many modern ideas and examples, workers in this field will inevitably find things to disagree with, and also errors. We are told on p222 that populations emphasising  $r$  selection typically inhabit regions with variable climates, such as the temperate zones, and on

I am afraid, however, that few biologists are mathematically adventurous. No doubt Professor Emery knows his audience at least as well as I do and caters for them appropriately. With a good, well-informed, teacher as a safeguard, this book might be successfully used as an elementary text, and many more sophisticated geneticists would be happy to have it in their pockets for ready reference.

Edmond A. Murphy

Edmond A. Murphy is Professor of Medicine at Johns Hopkins University, and Director of the Division of Medical Genetics at the Johns Hopkins University School of Medicine, Baltimore, Maryland.

p224 that most terrestrial vertebrates and perennial plants seem to be  $K$  selected, whereas most annual plants and insects are apparently  $r$  selected. The joint consequences of these two generalisations might well startle a biogeographer. Only later are we warned of the uncertainties of  $r$  and  $K$  selection theory.

Most of the errors I noticed were minor misprints, an omission of a text citation from the reference list, and so on. I regard the original meaning of "decimate" and the proper spelling of "repellants" (noun) as lost causes, but hope we may save *Rhagoletis* from being confused (p60) with the "frit" flies. More serious is the confusing blunder in Fig. 5-16, in which the prey curve is labelled "predator", and *vice versa*. On p229, the net reproduction rate  $R_0$  is said to be the number of surviving offspring per female: it should be female offspring. On p53, one of the equations for intensity of natural selection is badly misprinted.

Although the material, like the subject itself, is predominantly American, examples are taken from many parts of the World, including Britain. Some of the examples cited are difficult to present briefly, and in places (for example, pp137-8) the exposition is not clear enough.

To a British reader it seems odd to find no mention of life-table analysis and  $k$  factor analysis for diagnosing 'key factors' and density relationships among the successive mortalities in the life-cycle. If this (as it seems to me) is a serious omission, it is an exceptional feature in this generally comprehensive and well-balanced account. With its stimulatingly up-to-date treatment, I should think it worthy of a place in the short-list of books for students of population biology.

M. E. Solomon

M. E. Solomon is Head of the Zoology Section at Long Ashton Research Station, University of Bristol UK

## Feel for biological rhythms

*An Introduction to Biological Rhythms.* By J. D. Palmer. With contributions by F. A. Brown, Jr and L. N. Edmunds, Jr. Pp. xvi+373. (Academic: New York and London, August 1976.) \$19.50; £11.90.

THE author describes this as an introductory book, pitched at a level between popular books and technical treatises; these two stools are difficult to bridge. The enquiring student, to whom it is in part addressed, might find it difficult to evaluate for himself the evidence for many of the statements made: each chapter ends with some recommended further reading, but the text does not direct the reader specifically to the appropriate sources for the different assertions. This would indeed hardly be possible in a book with so wide a compass in such modest size.

Apart from general descriptions of the nature and properties of biological rhythms, circadian, lunar and annual, the author describes in more detail rhythms in unicellular and multicellular plants and animals, and the use of circadian clocks in animal, particularly in bird navigation, as well as some of the implications for man. The popular appeal is contributed in part by anecdotes of the background of some experimental investigations, but also by such journalistic epithets as "flamboyant" experiments and the "callous" decapitation of cockroaches. There is a similarly journalistic passage about the motivation behind human subjects for experiments, which in no way enhances the scientific value.

Little over half the book is written, however, by the author himself. It ends with two extended chapters, by F. A. Brown and by L. N. Edmunds, discussing the two rival theories: that biological rhythms depend essentially on subtle geophysical rhythms, or that they are due to an internal timing mechanism. Dr Brown's constant advocacy of the former hypothesis is familiar enough, but his chapter is the least readable in the book. A little of it is simply polemical, but the greater part consists of closely reasoned arguments demanding from the reader a highly critical attention. More than one interpretation can be put on many of the experiments described, but it is convincingly shown that a wide range of organisms can respond to a variety of quite small geophysical influences whose detection was hitherto totally unfamiliar to animal physiologists.

Dr Edmunds in the concluding chapter avoids a polemical adherence to the endogenous theory but considers

various possibilities of its mode of operation. Simple and convincing evidence is given that, at least in some insects, the rhythmicity is controlled by a part of the central nervous system operating by the release of a chemical substance, and that in mammals and birds the suprachiasmatic nucleus, operating by way of the pineal, plays a dominant or at least an important role. With regard to the fundamental question of the underlying circadian escapement, which has been shown to be present even in single enucleated cells, the three major theories are outlined: theories depending on a biochemical feedback loop, on transcription along

a DNA template, and on ions and ion transport in membranes. With the advantage of a limited field to cover, this chapter is perhaps the most successful in introducing biologists from outside this field to the major lines of thought; although in other respects, by virtue of the very broad coverage of different periodicities in such a wide range of organisms, the earlier chapters should be very helpful for those who want, as the author puts it, to get a "feel" for the subject. **J. N. Mills**

*J. N. Mills is Brackenbury Professor of Physiology at the University of Manchester, UK.*

## Animal response to environmental parameters

*Comparative Physiology of Animals: An Environmental Approach.* By Richard W. Hill. Pp.xii+656. (Harper and Row: New York and London, 1976.) £9.90.

AFTER years with no suitable text for an undergraduate course in animal physiology, we are now confronted with a succession of such texts and the problem of choice. This latest offering, by Richard W. Hill, is a very readable book intended for students beginning their study of the subject. Hill places his emphasis on the animal in its natural environment and on the physiological responses to the various environmental parameters rather than on the fundamental mechanisms underlying these responses. The physiological principles and relationships are presented accurately but primarily in non-quantitative and descriptive terms, and the book should appeal to readers who favour this approach.

The scope is ambitious since it embraces all animal groups aside from protozoans and metazoan parasites. The chapter by chapter organisation is based on physiological functions or environmental factors. The opening two chapters on organisms in the environment and on energy metabolism set the stage nicely for the following chapters on thermal relations, salt and water exchange, excretion, gas exchange and circulation. The book concludes with a series of integrative chapters on oxygen deficiency, high altitude, diving, exercise and rhythmicity. These final chapters are particularly successful in tying together the earlier material while reinforcing the environmental theme of the book. The coverage of the various

topics is comprehensive although occasionally somewhat repetitive (for example, the section of salt and water exchange). But students will appreciate the careful exposition of the concepts and the extensive use of appropriate experimental data illustrating the concepts. The more inquisitive students, however, may be frustrated by the fact that practically none of these experiments are referenced and most are not identified with the names of the investigators involved. Potential subscribers are therefore forewarned to put their own knowledge of the literature in order before assigning this text to their students. Some readers may also be troubled by the absence of any detailed discussion of the neural and endocrine systems, and the consequent patchy and superficial treatment of the role these systems play in the physiological response to the environment. In spite of these omissions, I found the overall organisation and content of the book to be quite coherent. It is an excellent text particularly suited for courses attempting to bridge the gap between physiology and ecology.

This book is not without value for more advanced readers as well. Hill's emphasis on the animal's response to its natural situation should remind those of us who study 'non-laboratory' animals in the laboratory to design our experiments carefully with the objective being to learn how these animals function in their natural milieu. Conversely, we must learn all we can of our particular animal's normal milieu in order to design useful experiments. These are also important lessons for students just beginning their studies in the field, and this book should serve that purpose well, while at the same time providing a sound introduction to animal physiology. **Donald C. Jackson**

*Donald Jackson is Associate Professor of Medical Science at Brown University, Providence, Rhode Island.*



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## Human information processing

*Memory and Attention: An Introduction to Human Information Processing.* (Series in Psychology.) Second edition. By D. A. Norman. Pp. xiii + 262 (Wiley: London and New York, 1976.) Clothbound £8.45; \$15.45; paperback £3.95; \$7.20.

DONALD NORMAN'S set of seminars on *Memory and Attention* have been useful to university teachers since they were first published in 1969. It is a pleasure to have a second edition, so adapted and extended that it is now virtually a new book. It is arguably also a different kind of book, since it is now probably the best and most cheerful introductory text one could recommend to a layman who wishes to understand how work on artificial intelligence and information processing is currently applied to psychological problems.

Among a large, mostly very recent, literature this book has distinctive virtues. First it is by a single author, who writes well. Second, it is short. Most importantly it is a good natured book, which seeks to be eclectic in appreciating the values of a wide range of different approaches. It retains enthusiasm without losing balance. It is a book to teach *with*, although possibly not a book to teach *from*.

Norman hopes that "the study of human information processing is becoming the study of the human" but aptly warns that "one must always be wary of comparisons of human psychology with any man-made device, most especially with computers. The human mind is not a computer, and the differences between the two far outweigh the similarities". Norman is also rare among workers in Artificial Intelligence in having a scholarly interest in the history of psychological problems. To quote one of his favourite authors, William James, he also has an appetite for the "more nutritious objects of attention" in psychology.

He is willing to accept the kinds of questions which students bring to psychology from their discursive reading, and while pointing out that some of them are unprofitable, is willing to discuss them seriously and in an illuminating way. Thus we find comment (and very useful references) on the topics of mystical experiences (Carlos Castaneda *et al.*), hemisphere differences (Ornstein *et al.*), mnemonic systems (an entire very useful chapter and excellent bibliography), pathologies of attention and of cognitive processes,

and even the art of juggling. To some extent these are embroidery on a serious text, and they do not occupy much space. But they are not mere decoration. They provoke useful commentary, and show a recognition that the enthusiasms which bring students to psychology need not be stifled, but can mature into more usefully directed intellectual curiosity.

It is easy to dismiss such topics as irrelevant to current empirical work, and to depress students by insisting that psychology has nothing to do with the study of human experience and is concerned instead with the description of "performance" or "behaviour". Norman's caveat for psychologists should be taken seriously by all students, and properly understood is an antidote to the depression felt by research workers who may feel that they are wasting time on the precise definition of trivial problems. He points out that human beings have been the object of their own attention for a very long time, and that perhaps "We cannot discover anything surprising . . . It would be peculiar if our careful study of thought processes led to results which are at odds with our intuition". But he further points out that the bridging of a gap between a nebulous appreciation of generalities and the precision of predictive mathematical theory is an exciting, and ultimately a satisfying, occupation for a scientist.

The work follows the format of the first edition in presenting related readings with commentary and further development. The chapter on attention is now extended by a discussion of recent work by Kahneman and Moray, with useful remarks on the problems in terms of Bobrow and Norman's own distinction between "data-limited" and "resource-limited" processing. The previous section on short-term memory has been extended to cover the Craik-Lockhead idea of levels of processing. There are excellent chapters on pattern recognition programs sensibly restricted to detailed analysis of the problems encountered with a *single* new system (Reddy and Newell's Speech Understanding system). A section on semantic networks forms part of an excellent chapter on representational systems. There is a good introduction to imagery, in the same vein.

The book is research oriented rather than didactic, and ends as it should with a set of problems outstanding (Unfinished Business). Not the least of its values to students and to lecturers is the care with which really comprehensive bibliographies are assembled and annotated.

**Patrick Rabbit**

*Patrick Rabbit is Lecturer in Psychology at the University of Oxford, UK.*

## Memories are made of this

*The Psychology of Memory.* (Basic Topics In Cognition Series.) By Alan Baddeley. Pp. xvii+430. (Harper and Row: London and New York, 1976.) Hardback £10.45; paperback £5.45.

AT LAST, a textbook on memory which one can recommend on its own, without three or four other volumes. The topic is a big one, ranging from the strange phenomena of clinical repression or 'photographic memory' at one end, to the effects of disrupting protein synthesis at the other. In the middle, there are groups of experimenters measuring the accuracy of memory in prescribed conditions; who are steadily building up the rules by which the system functions, whatever its physiological base. Even these groups are subdivided, some looking at associations between single items; some at memory for sentences and propositions; some at the similarities and differences of long-term and short-term retention; some at the importance of the particular form in which an event is encoded (roughly, perceived) at the time it occurred; and so on.

The difficulty is that each of these areas is still relatively unconnected with the others. All of them are advancing rapidly; it is hard enough to form a clear picture of the sense emerging from the mass of experiments in one field, without venturing into the others. Most books therefore concentrate on one sector, often doing it well. Yet the problem for the student or non-psychologist is that the picture he gets from such a book is one-sided. Even the slighter and more introductory texts often confine themselves to one approach, but merely replace detailed evidence by bald assertion. It is not surprising that even professional psychologists are sometimes unable to see the wood of progress for the trees of particular experiments.

Dr Baddeley's book, however, is different. It is not merely that his index mentions Freud as well as Ebbinghaus, Bartlett as well as Chomsky, acetylcholine as well as mnemonics. He could scarcely cover them all exhaustively; but the novel feature is that each field is mentioned appreciatively. His own most familiar area is perhaps that of long-term as against short-term memory, but he is sympathetic to all; and though the amount of space he

gives to each may get less as it becomes more remote from his own specialised expertise, the reader is left aware of every approach. Furthermore, the writing is beautifully lucid, and yet each point is based always on a well-selected experiment rather than generalities. He is also accurate, which cannot be taken for granted.

Such a book should appeal to anybody who is prepared to read nearly four hundred pages on memory; despite the clear and attractive style, it is all information rather than exhortation. Readers will therefore need to come to the book already convinced the subject is important. If, for example, they are doing an honours degree in psychology, or worried by the memory failures they meet in neurological practice, they cannot do better than pick on this book as their main source of information.

As favourable a review as this one is, some criticisms are still necessary as a guarantee of a proper standard. First, the sequence of topics is rather arbitrary, and one feels the chapters could be read in almost any order. This seems to stem from boredom with the conventional approach of starting with the arrival of a stimulus at the senses, and following it through. Perhaps this convention has some merit in the absence of any other organising principle.

Next, the distinction of short-term and long-term memory is surprisingly cavalier, and relies heavily on the very rapid forgetting of recent as opposed to more remote events. But this phenomenon, as has often been said, does not justify such a distinction. Dr Baddeley is now used to distinguishing far more than two kinds of memory; but some students will still be struggling with their tendency to parsimony, and could do with a clearer presentation of the reasons for abandoning a single homogeneous memory store.

Finally, the excellence of the book for teaching means that Dr Baddeley's own ideas have not been let loose. We could do with expansion of his brief suggestions about a modified interference theory, or about the role of talking to oneself for working memory during mental arithmetic. Perhaps a specialist monograph might follow? Yet this survey is itself a contribution to the subject since it reminds us all that research in this field has come a long way in twenty years.

Donald E. Broadbent

*Donald Broadbent was Director of MRC Applied Psychology Unit from 1958-74, and is now on the MRC External Staff at the Department of Experimental Psychology, University of Oxford, UK.*

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## Substance of demography

A GROWING interest in our species is reflected in the recent appearance of several general textbooks on demography. *Population: Analysis and Models* by L. Henry (Arnold: London, hardback £9.95; paperback £4.95) adds a valuable member to the company and demonstrates the continuing strength and vitality of the French contribution to these studies. Although in agreement with the translators that no demographer can be without a reading knowledge of French, they are to be congratulated on providing an excellent translation for those whose command of the language is imperfect. This is nevertheless an imposingly technical text, with considerable mathematics, although for the less numerate progress is eased by the usually clear and adequately explained steps. The text introduces a useful and interesting graphical tool, the Lexis diagram; elsewhere, however, more might have been explained by graphical presentation: the treatment, for example, of age-sex pyramids seems to be unnecessarily brief.

M. Henry's approach follows in general the now common 'longitudinal' study (developed by American and British demographers), the examination of data based on generations rather than the more limiting and older 'cross-sectional' method using only data from periods of a few years. The longitudinal approach is useful in enabling the link between population structure and movement to be clearly developed, and is well suited to the sociological emphasis of demography in the English-speaking world.

The description of the book on the jacket stresses that it covers the conventional substance of demography—source materials, methods of description, nuptiality, fertility, family formation and mortality (note the order!)—but also emphasises the author's attention to migration and its effective integration with population structure. Amid all these goodies, however, one dish seems inadequately prepared. There seems to be a general weakness among demographers that they give little depth to the spatial

elements of population—on p 13, under "Analysis of the Results", two meagre paragraphs on geographical distribution are so trite that they would have been better omitted. But even later in the text, especially when dealing with migration, the spatial element of which is so pronounced, there are statements over which geographers would readily take issue with the author. Regional variation in dynamic patterns seems to go inadequately recognised.

Part II, the final but lesser half of the book, is an introduction to demographic models and, perhaps importantly, a statement as to why models are of value and why they should be considered by demographers. Much of this section is centred round Lotka's theory.

The French version of the UN multilingual demographic dictionary adds a final phrase to the definition of demography that it is a study "from the quantitative point of view"—M. Henry has made just this point.

*Methods and Materials of Demography* by Shryock *et al.* (Academic: New York and London, \$16.50; £9.55) is a condensed version of a two-volume work of the same title originally published by the US Bureau of the Census in 1971 with subsequent later editions. It is now designed primarily as a textbook for courses on various aspects of demography, but it is equally useful to professional workers with occasion to understand or use demographic data for analytic studies.

The author of the abridgement claims to have presented the methodological material in the simplest mathematical form in order to make the text as useful as possible, a claim which even cursory examination of the text supports. For the most part a modest understanding of algebra and statistics will suffice to carry the user through the relevant sections.

Although originally a publication of the US Bureau of the Census, the illustrations and coverage are not solely restricted to American examples, but considerable weight is given to the problems of demographic assessment in the less developed countries. The author does, however, stress that the methods and measurements worked out for the USA are to a considerable degree "culture free" and may serve as well for any other country. In this condensation a necessary choice of inclusions and omissions had to be made, but a balance between formal demography and social and economic demography was sought. In this search, the author has been reasonably successful.

In general the step-by-step illustrations of examples are based on actual figures, although necessarily hypothetical statistics have had to be used in

some instances. Where official statistics have been used they have mainly been taken in their form as published, apart from a few instances of simplification. In this condensation, less emphasis has been put on historical development, the recommendations of international organisations and the practices of national statistical agencies, whereas the more technical and mathematical aspects of demographic analysis have been reduced in emphasis.

The book is, however, not without its weaknesses. Certainly chapter 5—Population distribution: Geographic areas—leaves many gaps, especially in the discussion of the important aspect of mapping distribution. The range of techniques examined is quite inadequate, and unfortunately some of the less impressive and informative methods are chosen for black-and-white line diagrams.

Nevertheless this is a fact-packed text invaluable to the do-it-yourself census maker, and brings together much material otherwise diffusely scattered. For any serious course on the principles of demography it should prove a useful if expensive textbook.

R. E. H. Mellor

R. E. H. Mellor is Professor of Geography at the University of Aberdeen, UK.

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● *Growing Points in Ethology* (Cambridge University: Cambridge), by P. P. G. Bateson and R. A. Hinde, has also been published in paperback, price £5.50. (For review, see *Nature* 265, 385, 1977.)

● In the review of *Environmental Chemistry* (Academic: New York and London; *Nature* 264, 818, 1976), the price was incorrectly quoted. This should read: \$15.95; £11.30.

## Comprehensive geomorphology

*Analysis of Landforms* by C. R. Twidale (Wiley Australasia: Sydney, New York, London and Toronto, £13.50; \$27) is intended for use as a text at advanced school level and for those taking degree courses in geography, geology, Earth sciences and environmental sciences. It sets out to provide a general text with an emphasis on Australia but with an adequate coverage of world examples.

The method of presentation leans heavily on photographs and diagrams and the book is lavishly illustrated. Yet its size is such that the written discussion is ample, as well as being of high quality and backed by discreet quantification. To keep the volume within manageable length, the nature and methods of close analysis are illustrated from a relatively few selected landforms, such as flared slopes, but here again the spacious text allows the general field to be covered in considerable detail.

The volume opens with a discussion of the scope, problems and methods of landform analysis and reveals a preference for multiple working hypotheses. The second part deals with structural geomorphology, including pseudo-structural forms; the third with geomorphological processes and climatic geomorphology; and the fourth with changes in time or historical geomorphology. The concluding part summarises the number and variability of factors involved in landform creation.

So it is better to travel hopefully than to arrive, but the student with this attractive companion will enjoy and benefit from the journey, especially as its ample text is strengthened by plenty of references and an enlightening geological appendix. The cost may seem high to those who have not seen the volume, but in fact its lavish visual aids and large-format pages make it good value. The printing—carried out in Singapore—is handsome and effective and adverse criticism must be minimal. It is misleading, however, to use Mercator's cylindrical projection for global distributions such as plate tectonics. This graticule has no uses whatsoever except for finding compass directions between localities, and students will never truly understand global distributions and movements until they see them on equal area projections.

Another recent book, *Geomorphology from the Earth* (Harper and Row: New York and London, £14.20), is a compact synthesis of geomor-

phology and stems from Chicago, an early citadel of landform studies. Its author is well known for his illuminating writings on the physical environment and its significance for Earth sciences ranging in diversity from archaeology to biology. He assumes—quite rightly—that an understanding of the surface forms and textures of our planet is a necessary ingredient of a liberal education; that landforms and the processes that create them are fascinating to scientists and curious people everywhere; and that persons of all ages and educational backgrounds gain from geomorphology a new appreciation of other environmental sciences. I have known students with souls so dead as to exclude empathy, males more curious about velocity than volcanicity, and females more interested in finery than feldspars, but have no intention of being an ancient mariner at a geomorphological feast for this is attractive fare laid out in an enticing way.

Professor Butzer keeps his aim of a general introductory text clearly in view and includes the essentials, critical interactions and brief overall perspectives. New findings and modern developments are incorporated throughout with a judicious use of mathematical expressions and, for readers who want more specialised aspects, with ample international sources in lists of references. The contents cover a broad field and, being confined to a moderate length, vary in intimacy of detail, the tendency being to elaborate most on forms and processes of greatest human significance. Commendably, space is found for a historical summary of modern concepts, before dealing with the lithosphere, weathering, soils and the sculpture of hillslopes. There follow discussions on topics such as fluvial dynamics and erosion, the work of glaciers and of waves and the importance of crustal movements. Separate chapters are devoted to periglacial landforms and to the characteristic landscape assemblages of mid-latitudes, deserts and tropical regions.

The high quality of the explanations, diagrams and photographs and the emphasis on the essential rather than the exotic will be admired by geomorphologists. Perhaps general readers may not appreciate the wisdom in the compilation but they will realise that geomorphology is a fascinating science and of great human significance. The text, in spite of its price, will also be valued as a stimulating revision for students already acquainted with the subject.

**Robert P. Beckinsale**

*Robert Beckinsale is an Emeritus Leverhulme Fellow and a Special Supernumerary Fellow of University College, Oxford, UK.*

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## Perspectives on physical geology

PHYSICAL GEOLOGY, defined by S. Judson *et al.* in *Physical Geology* (Prentice-Hall: Englewood Cliffs, New Jersey, \$14.50) as a discipline which "addresses itself to the composition, classification and origin of earth materials, and to the physical and chemical processes which go on at or beneath the earth's surface", covers a formidable range of material. At the small scale it deals with soil creep and at the other global tectonics. In addition it seems that under its umbrella one must attempt to cope with the study of resources, with environment, and with the characteristics of other planets.

It would be remarkable, given the scope that this offers, if three books on physical geology were to cover the same material in broadly the same way. Three recently published volumes however, are remarkable in just such a way. They have plainly been aimed at the same market (majors and non-majors in a one-semester or one-term course at the American college level); they have broadly similar format (multi-colour printing, many illustrations, good type); they all attempt to cover similar ground, and they all make commendable efforts to be comprehensible and attractive.

The differences between them are of a cosmetic rather than a fundamental nature. J. H. Zumberge and C. A. Nelson in *Elements of Physical Geology* (Wiley: New York and London, £7.20; \$14.40) offer the best colour illustrations of rock types and minerals together with an illustration of a seemingly topless Hawaiian surfer. J. E. Sanders *et al.* in *Physical Geology* (Harpers College, Harper and Row: London and New York, £10.45) offer flip drawings of Pangaea on the top corners of some 40 pages to "present the idea of drifting continents as vividly as possible", together with extracts from other writers (which they term "field trips"). And Judson *et al.* both pose and answer questions for the student, but (presumably why on a page for page basis they are cheapest) they offer slightly less glitter.

A second remarkable feature of such books would be for them to range successfully and adequately across the great range of material they seek to cover. Broadly speaking all of them do this, and broadly speaking their content and their guides to further reading are up-to-date. They all seek to put across the rudiments of mineral characteristics, rock types, volcanoes, weathering, mass movement processes, rivers, orogeny and plate tectonics.

Judson *et al.* use the plate tectonics concept as a framework for much of their discussion whereas it is less prominent in Zumberge and Nelson and in Sanders *et al.*

Inevitably there are inadequacies, omissions or lack of awareness of recent work. Rates of geomorphic processes are consistently neglected; climatic changes are given scant attention; coral reefs are accorded little prominence; Davis's great cycle of erosion is relegated to the very briefest of mentions; and the nature of water movement in drainage basins (particularly throughflow and runoff genera-

tion) is hopelessly out-of-date in the way that it is treated.

Overall there is not a great deal to choose between these three texts. They all offer clear, well-produced introductions to their views of physical geology. Probably their main use in Britain will be as texts for sixth-formers, but most University undergraduates would find shorter texts covering specialised topics at a greater depth more useful.

Andrew Goudie

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## Structural geology

*An Outline of Structural Geology* by B. R. Hobbs, W. D. Means and P. F. Williams (Wiley: New York and London, \$20; £10) is intended to replace Sherbon Hills' *Outlines* (Prentice-Hall, 1972) as a comprehensive text on structural geology for undergraduates. The involvement of three authors, all well known and experienced research workers, is perhaps a comment on the rapid growth in the complexity and diversity of the subject over recent years. Some years ago structural geologists were chided with scientific myopia, specifically, for having 'played' with minutiae while others discovered plate tectonics. Fair comment or not, this reminds us that when subjects develop toward a labyrinth of narrow specialisations there is an urgent need for a survey of the whole field of activity.

The plan of this book is not novel, indeed it has much the same lay-out as Sherbon Hills' and its success depends on the quality of treatment of familiar topics. It starts with mechanical aspects and stress-strain theory, and progresses from minor structures (microfabrics) through folds, faults, foliation and lineation to major structures and, finally, plate tectonics. The 'bridge' from small to large is in parts of a useful, but rather brief, chapter on "structural associations", that is thrust belts, block faulted regions, and so on. There is also an essential chapter on geometrical analysis and this topic, including stereographic analysis, is continued in an appendix.

It is a pleasure to record that the authors' surefooted approach, with its mingling of fact and comment, hardly ever lets them down. Fortunately, the authority which is evident in individual sections of the book is not spoilt by any overall lack of balance which is I suppose a danger when several authors are involved. Of course it is not perfect. For this reader, the splendid section on microfabrics is not always

matched elsewhere in the book and it is surprising to find so little on fluid pressures, or on pressure solution, or on techniques for estimating strain, shortening and displacement in orogens. My impression is that the authors are happier on the minor structures than with major structural phenomena. Their chapter on plate tectonics, however, is quite admirable.

The illustrations are vital in a book on structural geology and here there are many excellent diagrams and photographs, and very few disappointments.

The authors have designed their book for undergraduates. There are several potential rivals even if we exclude J. G. Ramsay's classic advanced book (*Folding and Fracturing of Rocks*, McGraw-Hill, 1967) and that by F. J. Turner and L. E. Weiss (*Structural Analysis of Metamorphic Tectonites*, McGraw-Hill, 1963), which has never been suitable at this level. The most recent editions of the respected books by L. U. de Sitter (*Structural Geology*, McGraw-Hill, 1964) and M. P. Billings (*Structural Geology*, Prentice-Hall, 1972) cannot be considered as truly modern in approach.

The books by E. W. Spencer (*Introduction to the Structure of the Earth*, McGraw-Hill, 1969) and P. C. Badgley (*Structural and Tectonic Principles*, Harper and Row, 1965) share some of the virtues of de Sitter's book in that they include 'potted' accounts of classic regions for the study of deformed rocks and this must be an asset for the hard pressed undergraduate. But the present book must surely take precedence over these because it goes further toward the goal of presenting structural geology, not only in modern terms but also as a coherent subject based on physicochemical laws. I think the research worker will also benefit from this reliable and well produced book.

M. R. W. Johnson

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## Accuracy, sufficiency and coverage in Earth sciences

THE three main qualities one looks for in a scientific dictionary are accuracy, sufficiency and coverage. The need for accuracy is self-evident, although due allowance must be made for the difficulty of encompassing within a limited space genuine differences of opinion over interpretations or even facts. Sufficiency, which refers to individual entries, is no less desirable. To describe a cat as a four-legged animal may be accurate but is hardly sufficient for someone not familiar with the beast. Finally, it is obviously desirable that coverage, which is dictated partly by the publisher who allocates the total space available and partly by the author who decides the length of individual entries, should be as wide as possible. Unfortunately, coverage and sufficiency are in mutual conflict and therefore demand compromise.

How, then, do three recently published works fare by the three criteria? *A Dictionary of Earth Sciences* (Macmillan: London and Basingstoke, £5.95), edited by S. E. Stiegeler, is a nicely produced hardback containing more than 2,500 entries supplemented by black-and-white diagrams. For a book described as a "personal reference work" and making no claim to comprehensiveness, this coverage is impressive. Unfortunately, however, it has been gained too much at the expense of sufficiency, for many of the entries are hardly adequate. To give but one example, the geomagnetic field is described simply as "The earth's magnetic field, which causes a compass needle to align north-south whatever its location on the earth's surface"; and although there are separate entries for "dipole field" and "non-dipole field" (not cross-referenced) the overall result is not very helpful. In this and many other, but far from all, cases one is left with all too little impression of what the subject is really about.

This dictionary would have been more successful if the number of entries had been reduced and the average length of them increased beyond the present 60-80 words. But that apart, the work has considerable merit. The general standard of accuracy is high, although there is an occasional fall (for example, in claiming uniformitarianism to be synonymous with actualism); and even the non-geologist would not find the text too formidable. Even ignoring the price differential, however, I have not been able to convince myself that this book is generally to be preferred to the *Penguin Dictionary of*

*Geology* (Penguin: Harmondsworth, Middlesex, 1972). The only reservation I have is that the Penguin book is weak on geophysics, a deficiency for which Stiegeler compensates. Perhaps the only answer is to possess both volumes.

As far as I know, *Earth Resources: A Dictionary of Terms and Concepts* (Arrow: London, £1.25), by D. Dineley *et al.*, is the first book of its kind to guide the general reader through a field of growing international concern. Its emphasis is rather different from that of the Stiegeler dictionary, although there is inevitably much overlap. A comparison of the terms common to both works is instructive, for it is clear that Dineley and his colleagues have been able to achieve a much higher degree of sufficiency by allocating themselves more space per entry—an advantage which applies equally to the many terms not covered by Stiegeler. The 900 or so entries presented here are thus generally informative and, as far as I can tell, accurate.

It is perhaps unfair to complain that a book fails to achieve what its authors never set out to do; but it is nevertheless a pity that the political, social and legal aspects of Earth resources have been largely neglected here. Admittedly there is a substantial entry on conservation; but, for example, asbestosis is not men-

tioned at all under the entry "asbestos", except indirectly by the single statement that "asbestos dust is considered to be a serious health hazard". And although there is a general acknowledgement of the existence of "international legal problems of ownership and mining rights of minerals on the deep sea ocean (*sic*) floor", there is not even a brief outline of the legal régime of the oceans. A fine opportunity of decreasing the artificial separation of science from its social context has thus been lost. As a "not too technical source of information" on the science itself, however, this book is indispensable.

Finally, *The Planet We Live On: Illustrated Encyclopedia of Earth Sciences* (Abrams: New York; New English Library: London, £22.50), edited by C. S. Hurlbut, is the most ambitious of the three, having a large format, 32 pages of colour plates, more than 550 diagrams and photographs and more than 1,800 entries. The coverage is wide and the degree of sufficiency high. But, alas, I must conclude that the whole enterprise is of little use, for the number of errors in the text is truly astonishing. Ignore it.

Peter J. Smith

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## Environmental dictionary

*The Environment: A Dictionary of the World Around Us*. By G. Holister and A. Porteous. Pp.280 (Arrow: London, October, 1976.) £1.25.

HOLISTER and Porteous have concerned themselves with a very broad multidisciplinary study area. As might be expected, they have not succeeded in providing an entirely satisfying compendium of the terminology in current use in the environmental management field. In fact, it seems that the published product may not be a dictionary at all, but rather an interesting and readable collection of bits of information, in a somewhat encyclopaedic format, on form and function within the ecosphere (a term not therein defined).

The authors have, of necessity, used an arbitrary system of choosing words or phrases commonly used in the disciplines of soil sciences, geology, hydrology, ecology, medicine, meteorology, chemistry, physics, biology, economics, engineering and cybernetics. But none of these disciplines is treated exhaustively with regard to environmental concerns, and the inter-

disciplinary conceptualisations of environmental management are inadequately organised and addressed.

*The Environment* is, however, loaded with terms and phrases which provide the curious with information and opinions about the quality, character and components of the environment. It should therefore provide the general reader with a working vocabulary suitable for improving his understanding of current environmental events in the newspapers, and the like. Because of its broad scope, the book is not suitable, therefore, as a technical reference manual for someone committed to serious research into environmental problems, and is by no means an exhaustive listing of the important concepts and terms to be found in the professional literature.

Some very topical issues are addressed in some detail. Nuclear power, agriculture and noise are treated in a particularly comprehensive fashion with good cross-referencing. The treatment of social science issues, such as environmental economics and the world futures modelling debate is somewhat less satisfactory.

Robert Otto

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## Astrotexts

THE popularity of astronomy as a university subject has increased enormously over the past decade or two, and fortunately the range and scope of astronomical textbooks has followed suit. Part of the secret of astronomy's popularity is that it can be studied on many different levels. It also contains something for nearly every student at university. It can be approached in descriptive, historical, philosophical and (unfortunately, usually the most important criterion) non-mathematical fashion. As such it can act as an excellent introduction to the disciplines and wonders of science for arts undergraduates.

The physical aspects of astronomy can also be stressed. In astrophysics the underlying physical principles of all planetary, stellar and galactic processes provide a potent example of the all-encompassing importance of physics. Also physical laws can be tested under extreme conditions of temperature, pressure, density, radiation and gravity. In astronomy these properties often have values far in excess of those obtainable in Earth-bound laboratories. A third approach is through astrogeology, a subject in which the student is brought into close contact with the recent data gleaned from the exploration of the Moon and planets by spacecraft. This subject opens up the fascinating prospect of comparing the geological features on the Earth's surface with those on Mars, Mercury, Venus and the Moon.

First, let us consider recently published books in the 'easy' category. *The Universe Unfolding* (Freeman: San Francisco and Reading, £9.50) by I. R. King, takes its place on the already groaning shelf of astronomy books for the innumerate. Its 504 pages contain

13 equations, nine of these being used in one figure, which explains the proton-proton chain of nuclear energy generation. The review questions at the end of each chapter are simplistic in the extreme: "What is the physical difference between gas and dust?"; "How big is the Earth? Answer this question in as many ways as you can", are two typical examples. The book cannot be criticised for its coverage, which is complete from absorption (interstellar) to zodiacal light, or its style, which is excellent, or its illustrations and figures, which are clear and well chosen, or the level at which it is pitched (14-yr-olds I would guess); but I do criticise it simply because it does not lead anywhere. The reader is not helped onward in his scientific investigations, and the foundations of astronomy, the observationally and mentally challenging science, are not laid. For my money, Paul Hodge's book *Concepts of Contemporary Astronomy* (McGraw Hill, 1974) still leads the field in the innumerate race.

Fred Hoyle's book *Astronomy and Cosmology: A Modern Course* (Freeman: San Francisco and Reading, £11.40) is pitched at the first-year undergraduate at university. Unlike King's book, which seems to be written as an end in itself, Hoyle's book is a stepping stone to more advanced astronomy. It is not so much a book for the innumerate but a book for the soon-to-be-very-numerate. In this it succeeds admirably. All that needs to be said is that "Hoyle does it again". Very few young professional astronomers today can say that they were not actively encouraged and mentally motivated by the problems posed in Hoyle's classic *Frontiers of Astronomy* (Heinemann: Melbourne, London and Toronto, 1955). His book, *Astronomy* (Macdonald: London, 1962), still stands as one of the most fascinating introductions to the history of astronomy.

*Astronomy and Cosmology* makes three in a row. In the preface Hoyle states that "The time has come for astronomy to take its place as a major branch of physics. Such is the point of view from which I have tried to write this book". So the book quite rightly stresses the underlying prime-movers of astronomical systems. It is written on two levels, each section being followed by an appendix which gives technical support to the main text and extends the discussion where Hoyle thinks it worthwhile. The excitement and fascination of astronomy shine through this book as, thank goodness, do some of Hoyle's own idiosyncrasies. One feels as though one is not only in the grip of a stimulating subject but also in the presence of a great mind. No science scholar or undergraduate should exclude this book from his reading list.

Now for something completely different. *Man Discovers the Galaxies* (Science History Publications: New York) by Richard Berendzen, Richard Hart and Daniel Seeley, is a story book. The story is about the development of our modern view of the Milky Way Galaxy and its relationship to neighbouring galaxies. It is a story with colourful characters—Shapley, Kapteyn, Curtis, Hubble and Einstein—conflict and debate (the memorable set-to between Shapley and Curtis before the National Academy of Sciences in 1920), and a mysterious ending, the continued discussion of relativistic cosmologies and the interpretation of redshifts. Using a large collection of original letters, photographs and archival treasures the authors have produced a rare thing, an astronomy book that cannot be put down. Many congratulations.

The relatively new field of astrogeology is represented by two worthy textbooks, *Planetary Geology* (Prentice-Hall: Englewood Cliffs, New Jersey, £14.35) by Nicholas M. Short and *Space Geology, An Introduction* (Wiley: New York and London, £11.50) by Elbert A. King. In a rapidly advancing subject like this, textbooks provide a good staging post before one gets embroiled in the argy bargy of recent publications. The books should encompass a series of reviews with references to the keynote papers, these forming useful points of entry into the vast literature. These two books by Short and King both succeed in doing this. King spends more time on meteorites, tektites and asteroids (obviously his speciality) than Short. Both emphasise the Moon, Short spending over 50% of his book discussing this enigmatic body, King only 30%. Mars is dealt with equally by both. I preferred the layout of King's book: each chapter is rounded off with a reference and note section and a comprehensive list of suggestions for further reading. Short only has about



Illustrations taken from *Herbal* by J. W. Krutch. Pp. 255. (Phaidon: Oxford, 1976.) Paperback £5.95. Left, Water Chestnut; right, Muskmelon.

200 references (half as many as King), all of which are lumped together at the back of the book (but thank goodness in alphabetical order—a much clearer format than King's numerical listing). Short contains a series of review questions at the end of each chapter, a god-send for those of us saddled with the task of setting tutorial and examination questions. There is little to choose between the two books but I would recommend Short's. His general approach seems more thorough and he makes much more use of graphs and line drawings. King's book, although excellently produced and illustrated, seems to be a little too full of glossy pictures and large print.

Finally we come to *Frontiers of Astrophysics* (Harvard University: Cambridge, Massachusetts and London, cloth \$20; paper \$8.95) edited by

Eugene H. Avrett. This is a very good book written for second- and third-year astronomy and physics undergraduates. The mathematics is not complex, but is not entirely omitted. Avrett has collected twelve, specially commissioned up-to-date reviews (of about 40 pages each) written by scientists distinguished in their chosen fields. Topics range from the Sun and the origin of the Solar System to star formation, star decay, neutron stars and black holes, infrared astronomy, interstellar medium, active galaxies, cosmology, and intergalactic matter. This book will be an invaluable source of information for many years to come.

David W. Hughes

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## Solid-state physics

THE author of a new textbook on this subject has to exercise ingenuity at the outset in finding a title that distinguishes it from its competitors. *Lectures on Solid-State Physics* (Pergamon: Oxford and London, £17.50) by G. Busch and H. Schade is based on lectures which Professor Busch gave for some years at ETH, Zurich. His students paid him the tribute of urging that the lectures be written up, and he credits his co-author with the actual task. The result is a sound and sensible book covering the topics one expects to find dealt with in the three or four years of an honours course in physics—essentially crystallography, X-ray diffraction, lattice dynamics, imperfections, the free electron model of a metal, electrons in a periodic potential, semiconductors, transport phenomena, and magnetism, in that order. Dielectric properties, and superconductivity have been omitted; in my opinion the latter at least is best left to a post-graduate course. The treatment is thorough; there are a few places where I felt the physics was obscured by the algebra but all-in-all this is a book which students would find very useful.

*Quantum Theory of the Solid State* (Academic: New York and London, £11.30; \$19.50) is a scholarly book by an author (J. Callaway) who has over the years made original contributions to most of the topics included. Although this is described as a Student Edition, the level of mathematical sophistication is decidedly too high for an undergraduate course, except possibly for theoretical physicists. Excellent bibliographies are given at the end of each chapter; they are pre-

dominantly to papers published before 1970, but with this book and use of the *Science Citation Index* a beginning research student could bring himself to the frontier of knowledge on any one of half a dozen topics in solid-state theory.

The two remaining books which the Editor sent me as Christmas reading are Numbers 4 and 7, respectively, of a series of monographs with the general title, *The Structures and Properties of Solids*. *The Electronic Structures of Solids* (Arnold: London; hardback £7; paperback £3.50) by B. R. Coles and A. D. Caplin covers those aspects of electrons in crystals that one finds also in a more comprehensive textbook such as that by Busch and Schade, but with greater emphasis on electrons in atoms and molecules by way of introduction. Students would find this approach helpful.

*Electron Microscopy in the Study of Materials* (Arnold: London; hardback £8; paperback £3.75) is a first-rate account of the subject by P. A. Grundy and G. A. Jones. Unfortunately there simply is not time for electron microscopy to feature in an undergraduate course in anything like this detail, but the book should be required reading for anyone who lectures on solid-state physics, and enterprising students will find it very interesting.

My experience has been that most undergraduates prefer a comprehensive textbook, and I have yet to be persuaded not to continue to recommend as the best buy C. Kittel's *Introduction to Solid-State Physics* (Wiley, £10.60), now in its fifth edition.

William Cochran

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## Stimulus for cosmological courses

*Principles of Cosmology and Gravitation*. By M. Berry. Pp. x+179. (Cambridge University: Cambridge, 1976.) Hardcover £7.00; softcover £2.50.

This is a purpose-written book intended to fill a gap in the literature. Cosmology, although one of the most popular and compelling sciences to the layman, is almost completely absent from science department syllabuses. The reason for this is that as general relativity is itself rarely taught, applications of the theory can hardly be expected. Michael Berry has made a laudable attempt at circumventing this problem by introducing as much of the essential ideas of cosmology without recourse to detailed relativistic formalism. Because of this necessarily superficial approach, this book will appeal mainly to physics undergraduates.

The happy circumstance that the universe appears remarkably homogenous and isotropic enables a superficial treatment to grasp the main results of modern cosmology, because the cosmic dynamics consists only of a simple rescaling of distances. The author does make a start at introducing some relativity theory. This is done clearly and directly, but may leave the reader caught between two camps, with insufficient knowledge to appreciate relativistic cosmology fully, but his faith in Newtonian theory and his classical physical intuition sufficiently shaken to cast suspicion on the later simplified ideas. Unfortunately, this is probably inevitable in what is really a disarmingly simple application of an exceedingly difficult theory. Dr Berry manages a careful balance between the two.

A chapter on black holes and massive objects is included, no doubt because of their strong popular appeal, but this is really a diversion. The main treatment concerns the standard cosmological models, and is presented in a straightforward and easy style. Information retrieval is good, the notation and exposition clear and economical.

This is a teaching book written in the tradition and spirit of H. Bondi's classic *Cosmology*, now over twenty years old. The level is slightly more advanced, but the readership substantially the same. I hope the author will succeed in stimulating new courses on cosmology in this country. Many problems and some solutions are included at the back of the book.

Paul Davies

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## Electromagnetism

USED the first edition of *Electricity and Magnetism* by B. I. Bleaney and B. Bleaney (Oxford University: London; hardback £17.50; paperback £9.95) as an undergraduate at Oxford in the fifties and when I came to Sussex in the sixties I bought the second edition. Now there is a third edition, which I have read with a sense of familiarity and which I still like, although I do wonder what sort of future it has for the student audience of the seventies. It is not cheap; but it contains a considerable amount of material likely to be taught in a range of undergraduate physics courses, rather than just one. In some ways this can be seen as a distinct merit; indeed, one wants to encourage students not to insulate one stream of knowledge from another. On the other hand, who would make *Electricity and Magnetism* their a main recommendation for a course on solid-state physics? And for a course on straight electromagnetism, who can overlook the range of cheaper texts which contain all the necessary material? Yet it is undoubtedly a valuable book, and one which in this new edition will continue to be referred to frequently.

The initial chapters on electrostatics and magnetic effects of currents are not greatly changed. They are still, it seems to me, a little dry and mathematical. The uniqueness theorem is no longer given but the section on expansion in special harmonics remains unpruned. The solid-state bias of the book has been strengthened and begins to tell as soon as currents are introduced in chapter 3. (There is a small misprint on page 65, which should read:  $\vec{r}^2 = 2\tau^2$ ).

Later chapters (11–13) are devoted to free electrons in metals, to the band theory of metals and to superconductivity. The arrangement of this material is greatly improved and the entirely new chapter on superconductivity is a good addition. The chapters on magnetic materials (6), on the various forms of magnetism (14–16) and on magnetic resonance (23) are not very different in this edition. The same is true for dielectrics (10). It is right that the space devoted in the previous editions to thermionic vacuum tubes has been severely cut back, and the progression from semiconductors (17) through solid-state devices (18, 19) to amplifiers and oscillators (20) and other applications is much more satisfactory.

The authors continue to use SI units, bringing in pascals in place of mm Hg, but failing to use joules (p466) in place of m.e.v. They are not consistent in the use of references; for example, why

are Oliver and Sucksmith referenced on p484 but not Henry on p450? Some chapters (for example, 14) are starved of references, although generally the record is good. There are nearly 200 problems, together with numerical answers where appropriate.

A second new book to appear in the past year, *Electric and Magnetic Fields* by Sir Charles Oatley (Cambridge University: Cambridge, London and New York; Hardback £9.75; paperback £3.50), is completely new although it has apparently grown out of a ten-year stint of lecturing at a non-specialist level to undergraduate students of all branches of engineering. The blurb claims that it would also be suitable for physics majors, but I doubt it. Physicists have to cover electricity and magnetism more thoroughly in a main course and do not generally have an introductory course followed by a specialist course. The author has tried to keep mathematics to a minimum but has not always succeeded. For instance, the section on the equivalence of a small plane current loop and a magnetic dipole is stiff with mathematics, whereas the idea of potential is introduced in an unnecessarily loose verbal way. These are perhaps details, but they exemplify an approach which makes me feel uneasy about the development chapters. The ordering and stress seem peculiar, right from page 1. In the very first paragraph of the

book we are assured that in wires it is "a reasonable assumption, at low frequencies, that the current is uniformly distributed over the cross-section . . . and concepts such as resistivity present no difficulty". A little further down we learn that the word "field" is to be used "in a general sense to indicate the region of space throughout which the effect which we are studying is appreciable". These statements strike me as questionable. Once the text moves into specific examples and applications, however, I begin to see its strengths.

The sections on induction and the chapters on the Laplace and Poisson equations and on non-linear magnetic materials are particularly valuable. Towards the end of the book, there is a chapter on electromagnetic waves, but this is too short (8 pages). The final chapter on the experimental basis of electromagnetic theory and some applications (such as eddy currents) is good. There are about 50 problems and a number of worked examples.

In conclusion, my own view is that for student audiences with which I am familiar (physicists, with a handful of chemistry and applied science majors), this book does not meet the challenge of Grant and Phillips' *Electromagnetism* (Wiley: London and New York, 1975).

D. S. Betts

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## Vector analysis

*Vector Analysis: A Physicist's Guide to the Mathematics of Fields in Three Dimensions.* By N. Kemmer. Pp. xiv+254. (Cambridge University: Cambridge, London and New York, January 1977.) Hardcover £12; paperback £3.95.

In the 'ideal university' this might be the right textbook for a physicists' first course on fields. But in most real universities there is not time to spend so long on one topic at this level. Students need to learn about partial differentiation, differential equations and vectors. They must also see how these mathematical tools are used in mechanics, in the description of waves and in thermodynamics. Electromagnetism and hydrodynamics provide the workshops where fields and vector analysis are to be understood, so we add half a dozen lectures to the 'Maths for Physics' course, to establish what the 'del' operator is, and we let the students get a feel for 'div', 'grad', and 'curl' later, when they need to use them. If there is to be a respectable minimum time spent on pure mathematics then it is hard to give all the tools of applied mathematics the un-

hurried treatment they may deserve.

Such problems are solved differently in each department, and Edinburgh University's solution has produced this very enjoyable book. As a course text, I would guess it covers about twenty lectures' worth of material, including Gauss' divergence theorem, Stokes' theorem, simple solutions of Poisson's equation and boundary behaviour. But it would be a difficult book to which to refer students from a briefer course on the same topics because its strength lies in the elegant geometrical approach, which Professor Kemmer develops in the first few chapters, to discuss lines, surfaces and volumes in Euclidean space. For a teacher this new notation means he must take the book whole or leave it alone. It could, however, be worth recommending as private reading to those stronger students who might be able to set aside the time to work right through it. The presentation is methodical and very clear, with excellent diagrams. There are large numbers of exercises at each stage and a full set of specimen answers at the back.

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